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STUDIES ON ASYNAPSIS IN THE ELM, ULMUS GLABRA HUDS.

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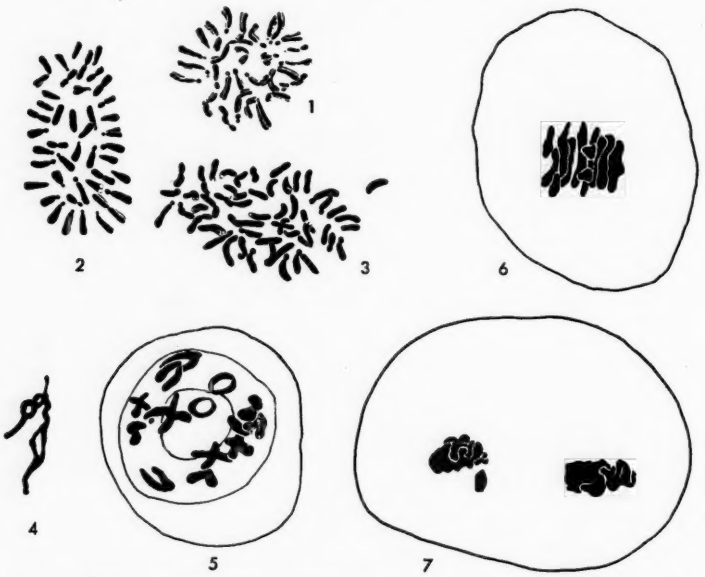
THE occurrence of asynapsis (*i. e.* decreased chromosome pairing at first metaphase) has been reported in several plant species since 1927, when LESLEY and FROST published their investigation of *Matthiola incana.* In 1943 a survey of species known up to that time was given by PRAKKEN. Since then further cases have been discovered, *e. g.*, in *Rumex* (LÖVE, 1943), *Musa* (DODDS, 1943), *Alopecurus myosuroides* (JOHNSSON, 1944), *Triticum* (LI, PAO and LI, 1945), *Phleum* (NORDENSKIÖLD, 1945), *Triton* (BÖÖK, 1945), and *Picea* (ANDERSSON, 1947). The present case, asynapsis in *Ulmus*, was discovered in 1943, further researches being conducted in 1944 and 1947 at the Forest Tree Breeding Institute, Ekebo, Källstorp, Sweden.

Material and methods. — Three asynaptic elms (Nos. 1, 7 and 10) from Kärrbogärde, Västergötland, and nine normal ones (Nos. 2, 3, 4, 8, 9, 11, 12, 13 and 14) growing at the same place were included in the investigation. One more normal elm from Fjärrestad, Skåne, was used in the crossing experiments. The morphology of tree No. 1 differed slightly from that of normal elms, the leaves being somewhat broader and darker, the branches thicker and the buds bigger; but these characteristics, indicating polyploidy, were probably due to environmental influences, since the chromosome number, determined in vegetative cells in the buds, was that characteristic of *Ulmus glabra*, *i. e.* $2n = 28$, and the mean length of the stomata did not differ from that of normal elms.

The origin of the Kärrbogärde trees is not known, but most probably they are of the same provenience as the other wild-growing elms in this district.

Branches were taken from the elms between December and February, forced in water culture in a greenhouse for 3 to 6 days, after which the buds were ready for fixation. Chrome-acetic-formalin was used as fixation fluid after prefixation with Carnoy. Staining was carried out with gentian violet, or in some cases with Feulgen. In both cases good results were obtained.



Figs. 1—3. Somatic metaphase plates of *Ulmus glabra*. — 1. diploid ($2n = 28$). — 2. triploid ($2n = 42$). — 3. tetraploid ($2n = 56$). — Figs. 4—7. Elm No. 2, normal. Meiosis in PMC's. — 4. diplotene pairs, separately drawn. — 5. early diakinesis, 14_{II}. — 6. I-M (all bivalents not visible). — 7. II-M.

To allow of the use of these asynaptic elms in crossing experiments other branches were grafted on 2—3 years elm plants, inserted in pots. The method used here — »bottle grafting», described in detail by JENSEN (1942) and by JOHNSSON (1945) — is generally employed at the Forest Tree Breeding Institute at Ekebo, Skåne, when working with species which have a specially short seed ripening time. The graftings and crossings were carried out in the greenhouse at the end of February, 1944, the seeds being sown immediately on maturing about a month later. Root tips of the young cross plants were fixed in chrome-acetic-formalin in the course of July—August; then cut into 14 μ sections and stained with gentian violet.

The pollen was stained with aceto-carmine. In measuring the diameters of pollen grains and the stomata lengths, a 40 $\times$ objective and a 15 $\times$ ocular were used, one unit of the scale equalling 2,4 μ.

The statistical analysis was performed according to BONNIER and TEDIN (1940).

TABLE 1. *No. of X:ta per bivalent in normal and asynaptic elms.*

Elm No.	Year	Stage	No. of bivalents	No. of X:ta	Mean No. of X:ta per bivalent
2 (normal)	1944	Diakinesis	350	358	1,02
		I-M	350	355	1,01
1 (asynaptic)	1944	Diplotene	264	320	1,22
		Diakinesis	103	108	1,05
		I-M	43	43	1,00
	1947	I-M	105	105	1,00
7 (asynaptic)	1944	I-M	114	114	1,00
	1947	Diplotene	127	136	1,07
		Diakinesis	82	86	1,05
		I-M	50	50	1,00
10 (asynaptic)	1944	I-M	259	259	1,00
	1947	Diplotene	113	127	1,12
		Diakinesis	58	65	1,12
		I-M	43	43	1,00

Somatic chromosomes. — In *Ulmus glabra*, as in most of the *Ulmus* species (TISCHLER, 1931; WALKER, 1932; SAX, 1933; D'AMATO, 1940), the somatic chromosome number is $2n = 28$. The lengths of the chromosomes vary, the longest chromosome pair being about 3,5 μ, the shortest one about 1,8 μ, and the rest about 2,0—2,5 μ. The centromeres are median (the long pair), submedian (2 pairs), the rest being subterminal or almost terminal. One pair of median length has a satellite connected to the short arm. The longest chromosomes and the satellite chromosomes are identifiable from cell to cell, but it is exceedingly difficult to distinguish between the other 24 chromosomes, their size being much the same and the position of the centromeres rather hard to determine (Fig. 1).

Meiosis in pollen mother cells of normal elms. — The early prophase has not been studied in detail, but at diakinesis there were 14 bivalents in each cell and 1,02 chiasmata (X:ta) per bivalent (Table 1, Fig. 5). About 79 per cent of the X:ta were terminal, but there was some difficulty in determining the position of the X:ta, and the value is probably too high.

At first metaphase (I-M) the number of bivalents was the same as

at diakinesis, or 14 (Fig. 6). The number of X:ta per bivalent was slightly decreased (Table 1), though the difference between this and that at diakinesis was not significant. About 74 per cent of the X:ta were terminal, and thus no terminalization seems to occur between the diakinesis and I-M. Ring bivalents were found in 23 cells out of 100, the mean number per cell being 0,26:

Number of ring bivalents	0	1	2	n
Number of cells	77	20	3	100

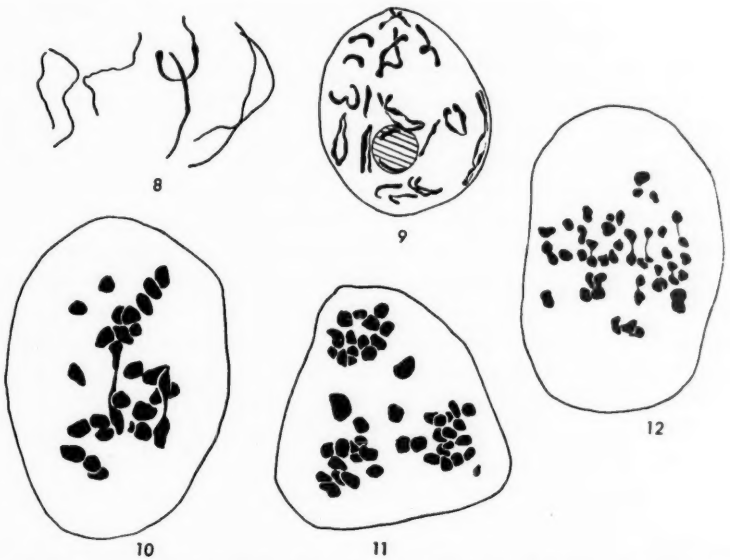
The later course of meiosis was normal (Fig. 7), the result being regular tetrads with the haploid chromosome number, $n = 14$. The mature pollen grains were of much the same size and shape (Fig. 20). The mean number of apparently good grains (*i. e.* grains well stained in aceto-carmine) in pollen samples from eight normal elms (1821 grains analysed) was 91,14 and the mean pollen diameter was $11{,}32 \pm 0{,}03$ units.

MEIOSIS IN POLLEN MOTHER CELLS OF ASYNAPTIC ELMS.

A. NUMBER 1.

Prophase. — No slides of early prophase were clear enough to permit detailed studies, the chromosomes being very long and thin and forming a network in the nucleus. In some PMC's, however, the separate threads could be followed throughout their length (Fig. 8). The pairing seemed to be fairly complete; unpaired threads were rarely seen, and in a few cells the chromosomes of a pair were separated but parallel. At diplotene, bivalents and univalents could be distinguished (Fig. 9). The mean number of bivalents per cell was 10,56, the maximum number in one cell being 14 and the minimum 6 (Table 2). The mean number of X:ta per bivalent was about 1,22, but this value is rather uncertain owing to the difficulty of determining the position of X:ta at this stage (*cf.* normal elms). Usually the univalents were lying two and two, *i. e.* in pairs, but without contact between the chromosomes. The distinction between this condition and prophase in the normal elm was clearly seen at this stage.

At diakinesis the number of bivalents per cell was considerably diminished (Table 2) and the univalents were scattered throughout the nucleus. The number of bivalents varied between 0 and 12 per cell, the mean number being 4,12. There was one, or occasionally two, X:ta



Figs. 8—12. Elm No. 1, asynaptic. Meiosis in PMC's. — 8. pachytene univalents and bivalents separately drawn. — 9. diplotene, 10_{II} and 8_{I}. — 10. I-M, 2_{II} and 24_{I}, the bivalents somewhat extended. — 11. II-A with a tripolar spindle. — 12. early II-A with dividing univalents.

per bivalent (Table 1). As at diplotene it was impossible to decide with certainty whether these were terminal or subterminal. The contraction of the chromosomes was as great as in normal elms, and since most of the chromosomes were of the same size and consequently rather uniform, there was no proof of any remaining pachytene pairing of the univalents as a rule, though there seemed to be a tendency in that direction (*cf.* RICHARDSON, 1935; BÖÖK, 1945).

TABLE 2. *Elm No. 1. Frequency of cells with 0—14 bivalents, in different years and different stages.*

Year	Stage	No. of bivalents per cell															Total No. of cells	Mean No. of bivalents per cell (M ± m)
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
1944	Diplotene							2	2	1	1	3	7	5	3	1	25	10,52 ± 0,44
	Diakinesis	4	4	4		3	2	3		1	1	1		2			25	4,12 ± 0,75
	I-M	133	160	192	150	90	41	14	5		2						787	2,16 ± 0,06
1947	I-M	36	35	17	9	1	1										100	1,05 ± 0,13

First metaphase. — At I-M the contraction of the chromosomes had further increased as in normal elms. The univalents were of a roundish shape, and the bivalents somewhat elongated. The latter always arranged themselves at the equator (*cf.* PRAKKEN, 1943; DODDS, 1943; HÅKANSSON, 1945), while the univalents were scattered in the spindle sometimes evenly, sometimes in two or three rather indistinct groups distributed on the two poles and the equator. On rare occasions all the univalents were grouped together at the equator (*cf.* LEVAN, 1938). Since the bivalents were uniform in shape, it does not seem to have been a case of earlier or later stages of metaphase. Thus the univalents showed no general tendency to submit to those forces which orientate the chromosomes in the equatorial plate, but to arrange themselves more or less at random in the spindle. The bivalents, on the other hand, behaved like those in normal elm.

The number of bivalents per cell was decreased still further but the mean number of X:ta per bivalent was about the same as at diakinesis (Table 2). Terminalization was complete in about 67 per cent of the whole. Only one spindle was formed, in contrast to, for example, the case found by BEADLE and MCCLINTOCK (1928) in *Zea*, where the bivalents were seen in a »head» spindle surrounded by some univalents, while the other univalents were found in one or more small spindles (*cf.* also BEADLE, 1930; EKSTRAND, 1932).

There was no dinstinct movement towards the poles when the I-A was due to begin. The bivalents remained at the equator, but in about one third of the cells the centromeres seemed to be pulled to some extent towards the poles, while the two halves were still kept together by the X:ta. For this reason the chromosomes were more attenuated than at full I-M (Fig. 10). This difficulty for the halves of the bivalents to separate at I-A was also observed in elms Nos. 7 and 10 (see below). ANDERSSON (1947) describes a similar case in asynaptic spruce, with the difference that fragments often occurred in the same cells as these threadlike bivalents. The explanation of this may be, as ANDERSSON points out, the occurrence of inversion bridges; but this assumption is probably erroneous, since inversion bridges do not occur in normal spruce and the common type of inversion bridges is not met with in asynaptic spruce. As regards the elms Nos. 7 and 10, where two interkinetic nuclei were usually formed, true inversion bridges were never found in No. 7 and only in less than 0,1 per cent of the cells in No. 10. The separation of the bivalent halves was pursued during I-A, though with some difficulty.

Split univalents were not seen in elm No. 1.

Since it was impossible to determine the distribution of the chromosomes to the poles in cells with two nuclei at interkinesis, little can be said about it. Probably, however, a group of univalents behaved as one unit and passed to the pole as if this unit were a single chromosome (*cf.* ANDERSSON, 1947).

Interkinesis. — A restitution nucleus was formed in about 79 per cent of the cells. In the remainder of the PMC's there were two nuclei (17,7 %), or in a few cells three or four (3,3 %). These were either of a roundish or a crescent shape. In cells with more than one nucleus the size of the nuclei varied: either there were two of equal size, or one big and one small nucleus or two big and one or two smaller ones.

Second division. — In PMC's with only one interkinetic nucleus any bivalents that were not separated as well as the univalents gathered at the equator at second metaphase (II-M), thus forming a regular metaphase plate. The univalents then divided and passed to the poles at II-A (Fig. 12), usually after the bivalent halves had separated and moved from the equator. The II-A was normal in 132 cells out of 150, resulting in dyads. In 14 of the remaining cells a tripolar spindle was found (Fig. 11), and consequently triads were formed, usually consisting of one big cell with 28 chromosomes and two small ones with 14 chromosomes each. The chromosome number, however, varied in a few of the cells; in one PMC the triads had the chromosome number 21 : 15 : 17, three chromosomes being lost in the cytoplasm. This distribution indicates non-disjunction of some of the chromosomes.

In cells with two interkinetic nuclei the second division was regular, but in the cells with more than two nuclei at interkinesis (about 3 per cent) the micronuclei seldom formed metaphase plates or, if they did, it was without subsequent separation of the chromosomes at II-A, since »tetrads» with more than four nuclei were never found, the chromosomes probably getting lost in the cytoplasm.

Another cause of this variation in the size of both tetrads and dyads was the lagging of chromosomes that occurred at II-A in about 50 per cent of the cells examined (*cf.* BEADLE, 1930; EKSTRAND, 1932; BERGNER *et al.*, 1934; LAMM, 1936; PRAKKEN, 1943). These lagging chromosomes, usually 2 to 6 per nucleus, were either included in the final nuclei or remained outside them and later disappeared.

As seen in Table 6, the diameters of the pollen grains in the three asynaptic elms varied between 11 and 21 units (one unit = 2,4 μ) and in the nine normal elms between 9 and 14 units. The average value

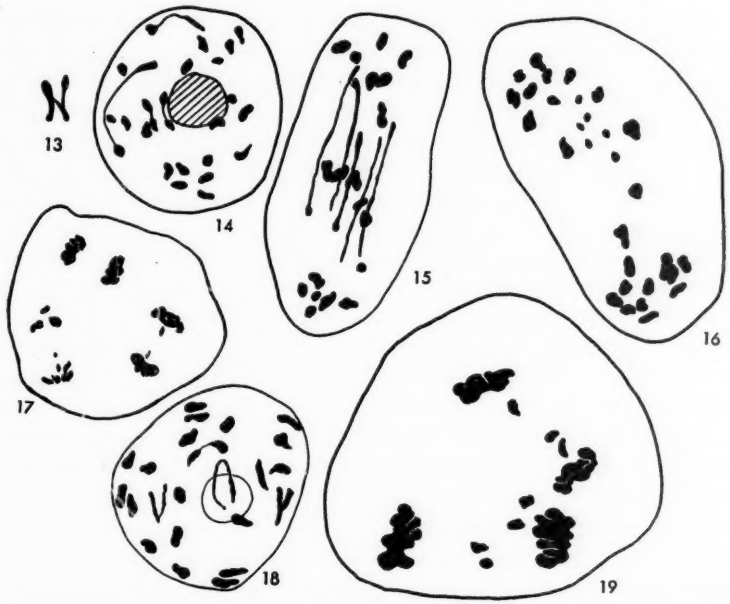
in the first group was 14,48 units and in the second 11,32 units. The difference between the two values is statistically significant. The variation in the asynaptic trees was about the same as in the normal ones, largely because of the fact that in the asynaptic elms only a few of the »good» pollen grains were haploid or aneuploid, the rest being diploid (Fig. 21).

Thus the meiosis in elm No. 1 is fairly stabilized in its irregularity and leads chiefly to the formation of »good» pollen grains with a diploid chromosome number.

B. NUMBER 7.

Prophase. — At this stage No. 7 differed from normal elms in the same way as No. 1. No plates at leptotene and zygotene could be analysed in detail, but at pachytene paired chromosome threads were clearly perceptible in most of the cells. Apparently X:ta or at least points of contact were formed between the two chromosomes of a pair. At diplotene, after the contraction of the chromosomes had proceeded further, both paired and unpaired chromosomes could be distinguished, but it was still extremely difficult to catch all the details of a whole chromosome set in the slides available. In the material from 1944 only 7 cells were analysable at diakinesis, the mean number of bivalents per cell being 4,29 and the maximum in one cell 7 (Table 3). If this average value is characteristic, at least to some degree, of the number of bivalents at diakinesis in No. 7, it agrees fairly well with that in No. 1.

New material was fixed in 1947 and, as Table 3 shows, the number of bivalents decreased from diplotene to diakinesis and from diakinesis to metaphase I, though the mean values for the two prophase stages were much lower than for the same stages in No. 1. In both No. 1 and No. 7, however, the average numbers of bivalents per cell at I-M were lower in 1947 than in 1944. Thus there must be a great variation in the number of X:ta formed from year to year, owing to external influences. If, on the other hand, the value obtained in 1944 at the diakinesis of No. 7 is not in accordance with the true condition, there must exist a true difference between the two elms either concerning the degree of pairing in early prophase or the time of separation of the previously paired chromosomes. Apparently the number of X:ta per bivalent was the same from diplotene to metaphase (Table 1), but the values obtained in the prophase stages were rather uncertain (*cf.* No. 1). The paired bivalents were often connected only by a thin chromatin



Figs. 13—17. Elm No. 7, asynaptic. Meiosis in PMC's. — 13. diplotene, separately drawn bivalent with clearly seen chiasma. — 14. diakinesis, 4_{II} and 20_{I}, the bivalent halves connected with long chromatin threads in two of the bivalents. — 15. early I-A, 5_{II} and 18_{I}, the bivalents extended. — 16. I-A, three of the univalents clearly split. — 17. late II-A in a cell with three nuclei at interkinesis; lagging chromosomes. — Figs. 18—19. Elm No. 10, asynaptic. Meiosis in PMC's. — 18. diplotene, 4_{II} and 20_{I}. — 19. late II-A, lagging chromosomes.

thread, sometimes drawn out across the whole nucleus (Fig. 14) — a condition reminiscent of the behaviour of the bivalents in some cells at I-M (see below).

TABLE 3. *Elm No. 7. Frequency of cells with 0—8 bivalents, in different years and different stages.*

Year	Stage	No. of bivalents per cell									Total No. of cells	Mean No. of bivalents per cell (M ± m)
		0	1	2	3	4	5	6	7	8		
1944	Diakinesis				2	3	1		1		7	4,29 ± 0,51
	I-M	43	101	116	94	45	18	3		1	421	2,16 ± 0,07
1947	Diplotene	6	11	9	13	1	5	5			50	2,54 ± 0,26
	Diakinesis	10	14	15	8	2		1			50	1,64 ± 0,57
	I-M	12	28	8	2						50	1,00 ± 0,14

First metaphase. — The pictures of I-M in elm No. 7 varied considerably, but the localization of the bivalents was always regular, since these chromosomes without exception were found at the equator forming a metaphase plate. As in No. 1, the number of bivalents per cell was decreased compared with that at the previous stages (Table 3). Cells with more than 5 bivalents were very rare. Ring bivalents were never found. The mean number of X:ta per bivalent was 1,0 (Table 1). Terminalization of X:ta in the bivalents examined had occurred in about 29 per cent in 1944 and 42 per cent in 1947. The univalents in cells containing bivalents were either evenly distributed along the spindle or appeared in more or less distinct groups at the two poles at the equator (*cf.* No. 1). Those which accumulated at the equator did not seem to take part in the metaphase plate formed by the bivalents, but were irregularly scattered between these.

The distribution of univalents to the polar and equatorial groups, respectively, at I-M was very irregular. Among 50 cells analysed, 4 had no univalents at all at the equator; 1 had, besides four bivalents, fourteen univalents in the metaphase plate, six at the one pole and none at the other. In 1 cell the univalents were evenly distributed in the spindle. In the remaining 44 cells there were from one to fourteen univalents in the middle group and from one to fifteen in the polar groups, the numbers six to eleven being the most frequent. In cells with no bivalents at all (about 10 per cent; see Table 3) only small groups of two or three univalents occurred, and no metaphase plate was seen, the groups lying scattered in the spindle. Occasionally one or two single univalents were found outside the groups, as in No. 1. It is evident that all these types of chromosome distribution are not different stages of one single type, but occur simultaneously in different cells (*cf.* above).

First anaphase. — In contrast to that of No. 1, the I-A was clearly differentiated from the I-M. The members of the bivalents separated and passed to the poles, joining the univalents and grouping there at I-M (Fig. 15). At the same time the univalents in the equatorial group migrated to the poles. In some of the cells the univalents at the equator had divided, but the chromatids were kept together by the undivided centromeres. Only in 5 PMC's in one flower were clearly split univalents found (Fig. 16), the number varying from 1 to 6 per cell (*cf.*, *e. g.*, BEADLE, 1930; EKSTRAND, 1932; LAMM, 1945; HÅKANSSON, 1945; ANDERSSON, 1947). In all these 5 cells bivalents were present (Fig. 3). With one exception both the chromatids passed to the same pole, thus

behaving as one unit. In one of the cells a univalent seemed to have split earlier, as one of the chromatids was found lying near the one pole while the other had moved a bit from the equator in the opposite direction ahead of the solitary bivalent. The other divided univalent in this cell was seen near the bivalent.

The distribution of the chromosomes to the poles at late I-A was as follows:

8:20	9:19	10:18	11:17	12:16	13:15	14:14	No. of cells
1	1	4	4	9	8	9	36

Occasionally a tripolar spindle was seen, and sometimes one or two chromosomes were found outside the spindle. In about 50 per cent of the cells examined, the bivalents tended to be stretched into thin threads before separating (*cf.* elm No. 1).

Interkinesis. — At interkinesis there were generally two nuclei of a size that varied slightly owing to the different chromosome distribution at I-A. Out of 300 cells examined 89 per cent had two nuclei, 4,7 per cent only one nucleus and the rest, 6,3 per cent, three nuclei. Thus a restitution nucleus was raised in only a few cases. The cells with three nuclei certainly had passed through a more irregular I-A, either with a tripolar spindle or with lagging univalents which never reached the poles but formed interkinesis nuclei of their own, like those chromosomes which from the beginning were excluded from the spindle, remaining in the cytoplasm during the whole stage. Consequently, the size of the nuclei in these three-nucleated cells varied considerably. More than three nuclei were not seen, which is rather surprising considering the type of irregularities at I-A. As pointed out above, however, there was a marked tendency for the univalents to gather in groups as early as I-M, and therefore a *group* of univalents rather than solitary ones was excluded from the anaphase movement, giving rise to one extra nucleus only. The single chromosomes seen in the cytoplasm, both at I-M and I-A, remained there as chromatin lumps and did not form true nuclei.

Second meiosis. — The distribution of chromosomes on the two plates at II-M was examined in 50 cells:

8:20	9:19	10:18	11:17	12:16	13:15	14:14	No. of cells
2	2	2	7	7	12	18	50

It agreed fairly well with the distribution at late I-A. The variation was slightly greater, but since only a small number of cells were

examined this difference is not significant. The percentage of cells with the distributions 12 : 16 and 14 : 14 was nearly the same in both stages (72 per cent at I-A, and 74 per cent at II-M). The chromosomes gathered regularly at the equator and formed metaphase plates. The extra chromosomes lying in the cytoplasm, however, did not seem to take part in any movement and were probably lost later on, since »tetrads» with more than four cells were very rare (6,5 %). Because of the paucity of cells with clearly divided univalents (only found in 5 cells in one flower at I-A) the behaviour of these divided univalents could not be studied in the second meiosis. Probably they did not interfere with the normal course of the process, since both chromatids usually went to the same pole, and very likely passed to the metaphase plates among the other chromosomes and later on separated at II-A (*cf.* BEADLE, 1930; LAMM, 1936; ANDERSSON, 1947). In the restitution nuclei the chromosomes formed one large metaphase plate, followed by a regular anaphase and resulting in dyads. This happened in every such cell studied, and monads were never found. In about 40 per cent of the cells, one or two chromosomes were slightly lagging (Fig. 17).

As was to be expected from the chromosome distribution at I-A and II-M, and from the regularity of II-A, most of the PMC's (83 per cent) contained four nuclei at the end of meiosis, though the shape and size of the nuclei naturally varied owing to their different chromosome number. This variation, however, was not especially marked, since more than 70 per cent of the nuclei contained 14 ± 2 chromosomes.

The pollen grains showed a rather variable picture (Fig. 22). In the first place, the number of apparently good grains was only 44 out of 220, or 20 per cent, the rest being more or less shrivelled and empty. Secondly, their diameters ranged from 11 to 21 units, the mean size being $14{,}75 \pm 0{,}02$ (Table 6). In elm No. 1, where almost exclusively

TABLE 4. *Elm No. 10. Frequency of cells with 0—8 bivalents, in different years and different stages.*

Year	Stage	No. of bivalents per cell									Total No. of cells	Mean No. of bivalents per cell (M ± m)
		0	1	2	3	4	5	6	7	8		
1944	I-M	41	85	80	70	40	16	10	1	2	345	$2{,}23 \pm 0{,}08$
1947	Diplotene	5	12	11	11	9	2				50	$2{,}26 \pm 0{,}20$
	Diakinesis	17	17	9	5	2					50	$1{,}24 \pm 0{,}15$
	I-M	19	22	8	1	1					50	$0{,}86 \pm 0{,}12$

TABLE 5. *Results of crossing experiments.*

Combinations	Cross No.	Parents ♀	Parents ♂	No. of clusters pollinated	No. of seeds obtained	No. of full seeds	Mean seeds per cluster	Proportion of full seeds	No. of plants obtained	2*n* (No. of plants examined)
as. × as.	1	Elm No. 1	Elm No. 7	210	564	5	2,69	0,89	2	42 (2)
	2		10	146	832	3	5,7	0,36	1	42 (1)
	3	7	1	168	32	—	0,19	—	—	—
	4		10	155	153	36	0,98	23,53	16	56 (15); 42 (1)
	5	10	1	158	48	—	0,3	—	—	—
	6		7	136	7	—	0,05	—	—	—
			Total	973	1636	44	1,68	2,69	19	(19)
as. × norm.	7	Elm No. 1	Fjärestad	100	147	35	1,47	23,81	12	42 (5)
	8	7		56	14	6	0,25	42,86	—	—
	9	10		55	84	36	1,5	42,86	10	42 (5)
			Total	212	245	77	1,16	31,43	22	(10)
norm. × as.	10	Fjärestad	Elm No. 1	108	886	468	8,2	52,82	72	42 (5)
norm. × norm.	11	Fjärestad	Elm No. 19	50	546	—	10,92	—	108	28 (5)
	12	Elm No. 2	Fjärestad	2	26	—	13,0	—	17	28 (5)
			Total	52	572	—	11,44	—	125	(10)

TABLE 6. *Pollen size and proportion of »good» pollen grains in normal and asynaptic elm.*

Tree No.	% »good» pollen	Pollen diameter (units)													No. of grains	Mean	$\frac{s}{x}$
		9	10	11	12	13	14	15	16	17	18	19	20	21			
Normal 2	96,61	7	96	96	1										200	10,41 ± 0,04	
3	95,18		41	115	43	1									200	11,02 ± 0,05	
4	95,92		1	14	49	31	5								100	12,25 ± 0,08	
8	92,74		1	49	67	3									120	11,60 ± 0,02	
9	92,79	1	15	66	16	2									100	11,03 ± 0,07	
11	95,27		3	29	51	17									100	11,82 ± 0,07	
12	56,23		3	27	45	24	1								100	11,93 ± 0,08	
13	71,32		8	41	36	12	3								100	11,61 ± 0,09	
14	87,30	2	6	54	34	4									100	11,32 ± 0,07	
Total		10	174	491	342	94	9								1120	11,32 ± 0,03	0,08
1	79,72				4	17	93	75	11						200	14,36 ± 0,06	
7	20,00			1	2	9	26	33	15	10	3			1	100	14,75 ± 0,02	
10	10,50			2	7	13	41	22	10	5					100	14,24 ± 0,09	
Total				3	13	39	160	130	36	15	3			1	400	14,48 ± 0,06	0,08

dyads were formed, giving rise to pollen grains with diploid constitution, the mean pollen diameter was 14,36 units, or very much the same as in No. 7. Thus the bulk of the »good» grains in No. 7 were probably diploid or nearly diploid, especially as the average value of normal haploid pollen grains is $11{,}32 \pm 0{,}08$ (Table 6). In general only the diploid pollen grains functioned, judging from the chromosome number of the plants resulting from the crossing between asynaptic elm and normal elm (see below). However, the number of F_1 plants was small, and vital haploid pollen was certainly formed, as will be seen from the fact that triploid plants arose from the crossings between asynaptic and asynaptic elms (Table 5; *cf.* however below).

C. NUMBER 10.

In the main the meiosis of elm No. 10 agreed with that of No. 7 and will not be described in detail here, though some facts may be of interest.

Prophase (analysed in the material fixed in 1947). — In stages previous to diplotene no PMC's could be analysed thoroughly, but at diplotene the number of bivalents per cell was estimated at $2{,}26 \pm 0{,}20$ (Table 4). It decreased at diakinesis to 1,2. The number of X:ta could not be determined with certainty (*cf.* No. 1 and No. 10), but generally there was one or on rare occasions two. When the X:ta were terminalized the paired members of a bivalent were sometimes connected only by a thin chromatin thread varying in length (*cf.* No. 7).

First metaphase (analysed in material fixed in 1944 and 1947). — The bivalents gathered in an equatorial plate, while the univalents showed the same tendency as in No. 7 to form groups outside the plate, though these were less pronounced. The number of bivalents per cell was $2{,}23 \pm 0{,}08$ in 1944 and $0{,}86 \pm 0{,}12$ in 1947. The difference between these two values is statistically significant ($t = 3{,}95^{***}$) and can only be due to the variation of external influences during the different years. The number of X:ta per bivalent was 1,0 or the same as in No. 1 and No. 7.

First anaphase and interkinesis. — The I-A was clearly distinguished from the I-M, as in No. 7. The univalents passed to the poles at the same time as the halves of the bivalents separated and moved in opposite directions. In about 80 per cent of the cells the bivalents were stretched into thin threads, these showing difficulty in separating. As mentioned above, the same phenomenon was observed in No. 1 and No. 7. This fact, however, did not interfere with the proceeding

anaphase movement: non-disjunction did not occur, the result being an interkinesis with two well-separated nuclei of almost equal size. There were some exceptions from this rule. A very small number of real chromatin bridges and fragments were found, indicating previously formed inversions (*cf.* DARLINGTON, 1937, a. o.). And occasionally (in 1,67 per cent of the cells) a restitution nucleus, generally of dumb-bell shape, was seen. Micronuclei were sometimes observed, originating from the chromosome groups which remained outside the spindle during I-A. The number of nuclei per cell at interkinesis was as follows:

No of nuclei	1	2	3	4	Total
No. of cells	5	281	14	—	300

Second division. — The distribution of chromosomes on the two metaphase plates in 25 cells was as follows:

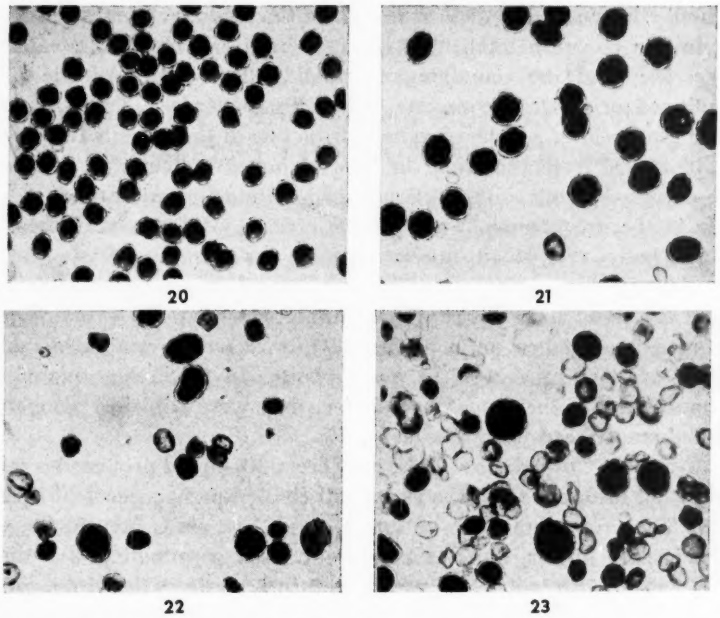
8:20	9:19	10:18	11:17	12:16	13:15	14:14	No. of cells
2		1	5	22	18	30	78

In about 40 per cent of the cells 14 chromosomes had passed to each pole, and with about the same frequency one pole got two or four chromosomes more than the other.

The metaphase plates were regular even in cells with one or three nuclei, but the anaphase went on rather irregularly with slightly lagging chromosomes, in contrast to normal elm, where the movement is simultaneous for the whole set of chromosomes (Fig. 19). Very rarely three or four poles were formed, causing further irregularity in the final chromosome distribution. Small chromatin pieces (fragments?) were seen in the cytoplasm in most of the cells, but were never present in any metaphase plate, nor did they show any anaphase movement, and certainly they disappeared at the end of meiosis. Chromatin bridges at telophase were occasionally found, with or without fragments.

Tetrads were formed in 87 per cent, dyads in 5,7 per cent and triads, pentads, or hexads in the remainder (300 cells studied). Each of the 8 triads found consisted of one large and two smaller nuclei, whereas 2 had two large and four smaller ones. Finally, 1 cell had all six nuclei of different sizes.

Only 10,5 per cent of the pollen grains were apparently good ones (Table 6); the mean diameter of these was $14{,}24 \pm 0{,}09$ units, which indicates a diploid constitution in most of the grains (Fig. 23).



Figs. 20—23. Pollen grains. — 20. Elm No. 2, normal. — 21. Elm No. 1, asynaptic. — 22. Elm No. 7, asynaptic. — 23. Elm No. 10, asynaptic.

MEIOSIS IN EMBRYO-SAC MOTHER CELLS OF ASYNAPTIC ELM.

Diakinesis was studied in one cell (28 univalents) and I-M in two cells (one bivalent each) from elm No. 1. They showed the same general appearance as the pollen mother cells, with the bivalents in the equatorial plate and the univalents distributed in the spindle at I-M.

RESULTS OF CROSSING EXPERIMENTS.

Asynaptic × *asynaptic.* — A high degree of sterility was characteristic of the asynaptic elms when crossed with each other. The number of seeds per cluster was small compared with normal elms (Table 5), the mean value per crossing ranging from 0,05 to 5,7 per cluster. Mostly the seeds were empty, with one exception: the cross Kärrbogärde 7 × 10. Generally in normal elms about 30 per cent of the seeds are

without embryo. A little less than half of the seeds germinated, producing in due course a total of 19 plants. Of these, 15 were tetraploids ($2n = 56$), and the remaining 4 triploids ($2n = 42$). All the first-mentioned originated from one cross (Kärrbogärde 7×10). In the latter group either a reduced gamete from one of the parents fused with a diploid one from the other, or intermixing of pollen from a normal elm occurred owing to the difficulty of isolating flowering twigs effectively in the greenhouse. Three of the crosses yielded no full seeds at all and had a very small number of seeds per cluster.

Asynaptic $\times$ *normal.* — The number of seeds per cluster was about the same as in the previous combinations, but the proportion of full seeds was higher, or 31 per cent. Only 22 seeds germinated (about 25 per cent) and produced as many plants. In 10 of these plants the chromosome number was $2n = 42$, *i. e.* they were triploids. One cross yielded empty seeds only (Table 5).

Normal $\times$ *asynaptic.* — The only cross attempted produced a result somewhat different from the reciprocal cross, Kärrbogärde $1 \times$ Fjärrestad. The proportion, both of »good» seeds and seeds per cluster, was considerably higher, but the number of seeds germinating was somewhat lower. 72 plants were raised, and in 5 of these the chromosome number was determined, this being in each case $2n = 42$. The remaining 67 plants were triploids, too, judging from the lengths of their stomata. It would seem, therefore, that only the diploid pollen grains from Kärrbogärde 1 functioned in this case. Presumably all the vital egg-cells of No. 1 were diploid, too, and therefore the reduced gametes that must have functioned in the crosses 1 and 2 (see Table 5) might have come from the male parents, *i. e.* elms Nos. 7 and 10.

Normal $\times$ *normal.* — Finally, 2 crosses were made between three different normal elms, the number of seeds per cluster being still higher than in the other crosses. Unfortunately the proportion of full seeds was not determined, but in normal elms this generally amounts to about 70 per cent. Out of 572 seeds 125 plants were raised, all of which were diploids judging from the stoma lengths. Root tips were fixed from 10 plants, and the chromosome number was found to be $2n = 28$.

The number of plants raised from the 12 crosses was, however, very small; probably different values could have been obtained in the asynaptic $\times$ asynaptic crosses and perhaps also in asynaptic $\times$ normal and reciprocal, had the progeny been more numerous. But the fact remains that the fertility is reduced in asynaptic elms, as in most other asynaptic plants of different species. Probably only diploid or haploid

pollen grains function, all aneuploid ones degenerating (*cf.* BEADLE, 1930, 1933; EKSTRAND, 1932; BERGNER *et al.*, 1934).

MORPHOLOGY AND ANATOMY OF PROGENY WITH DIFFERENT CHROMOSOME NUMBERS.

Morphological characteristics. — The diploid and triploid plants were very similar, though in the latter the leaves seemed slightly more varying in shape and were perhaps a little more sharply dentated.

The tetraploids, on the other hand, had sharply dentated leaves of a dark green colour and of very varying form. The leaves were small, almost dwarfish in size. As regards the plant heights, there were true differences between tetraploids on the one hand and diploids and triploids on the other.

$2n$	28	42	56
Mean height	$28{,}30 \pm 1{,}49$	$29{,}30 \pm 1{,}81$	$11{,}69 \pm 1{,}34$
No. of plants	10	33	16

Thus the tetraploids grew more slowly than diploids and triploids, the development of leaves and stem being retarded. In *Populus*, JOHNSSON (1945) observed that the stems of triploid aspen yearlings, unlike the leaves, did not show any gigantism, and stated: »It is a very common observation that the polyploids develop more slowly than the diploids». In *Ulmus* this slower development was not observed until the chromosome number in the plants had increased to four times the haploid one. Now, in trees the growth continues for many years, and it may be assumed that the tetraploid plants later on overtake and outgrow the diploids and triploids (*cf.* JOHNSSON, 1945). On the other hand, it is also commonly observed that in many species (and genera) there exists an optimum chromosome number and that plants with a higher number of chromosomes are less vital. In the present investigation all plants examined were about 7 months old; consequently nothing can be said about their later appearance.

Stomata and pollen. — The length of the stomata was determined on 10 diploid, 31 triploid, and 14 tetraploid plants. From each plant three leaves were taken: one from the top, one from the middle part and one from the base of the plant, the last-mentioned being the smallest. 50 stomata per leaf were measured, the relevant data being given below:

$2n$	28	42	56
Mean length (units) . . .	$13{,}20 \pm 0{,}01$	$15{,}19 \pm 0{,}002$	$17{,}35 \pm 0{,}004$
No. of stomata	1500	4650	2100

The difference in stomatal length between plants with different chromosome numbers is highly significant. The inter-plant variability is more pronounced in tetraploid plants than in others. This phenomenon, *i. e.* greater variability of stoma lengths in tetraploid plants, was also found by LAMM (1945) in *Solanum*, but there the effect may have been partly due to the colchicine treatment employed.

Pollen samples were taken from the 3 asynaptic elms and from 9 normal ones (see Table 6). The diameters of between 100 and 200 apparently good grains were measured in each sample. The grains were roundish or slightly oblong in shape, and there was no apparent difference between diploid and polyploid pollen grains except in size (Figs. 20—23).

There was, however, a significant difference in mean pollen diameter between normal and asynaptic trees (Table 6), owing to the fact that pollen grains from the asynaptic elms probably had a diploid condition. As regards the asynaptic group, the variability in No. 1 was less marked than in Nos. 7 and 10. The cause of this must be that in No. 1 73 per cent of the PMC's formed regular dyads instead of tetrads in the second division, thus giving most of the pollen grains the diploid chromosome number. In the other two elms the formation of »tetrads» was far more irregular, as mentioned above.

DISCUSSION.

The three cases of asynapsis described in the present paper seem to be of the same type. The main course of meiosis ran as follows:

Normal or nearly normal pairing in pachytene, but probably decreased X:a formation — a varying number of the paired chromosomes disjoining during diplotene and diakinesis — a varying number of bivalents in I-M — a more or less irregular I-A — interkinesis with one, two or (in a few cases) more nuclei — in the main regular second division resulting in microspores of varying number and size.

There were, however, some differences between No. 1 on the one hand and Nos. 7 and 10 on the other. The proportion of cells with only univalents at I-M was higher in No. 1 (16,9 per cent) than in the others (10,2 and 11,88 per cent respectively; see Tables 2, 3 and 4).

The tendency for the univalents to appear in groups at I-M was slightly less distinct in Nos. 7 and 10. The greatest difference was found at I-A and interkinesis. In No. 1 the anaphase movement was retarded and a restitution nucleus was formed in about 80 per cent of the PMC's, whereas in Nos. 7 and 10 only 4,7 and 1,7 per cent respectively of the cells had a single-nucleated interkinesis. The number of bivalents per cell seemed to be immaterial in this connection, for in elm No. 1 there were about five cells with a restitution nucleus to every one with univalents alone; whereas in the other two elms the proportion of cells with one nucleus at interkinesis was less than half that of cells without bivalents (*cf.* ANDERSSON, 1947). These circumstances were reflected in the tetrads, elm No. 1 having about 73 per cent of cells with two microspores, the other about 6 per cent. In the former, however, no more than 4 microspores were ever observed in one cell, while in the latter some cells were found with 5 or 6 microspores.

These differences, however, are probably due to different intensities of the same type of asynapsis, the separation of the paired chromosomes being less rapid in the prophase of No. 1 and the anaphase movement terminating earlier.

Cases of reduced pairing at I-M have been discussed earlier, *e. g.*, by BEADLE (1933), PRAKKEN (1943) and JOHNSSON (1944). PRAKKEN arranges all cases known in 1943 into six groups, the first of which is described as: »Asynapsis due to the action of a distinct gene or genes (or some slight structural change)». Most probably the type of asynapsis found in the elms from Kärrbogärde belongs to this group, since there is no reason to assume structural hybridity or hybrid origin.

This group is subdivided into three »intensity series» based on the number of univalents at I-M: (1) Weak asynapsis with a few univalents in some of the cells. (2) Medium—strong asynapsis with many univalents in most of the cells. (3) Complete asynapsis with univalents only, or some rare bivalents. The present case may be assigned to the second series, *i. e.* there are many univalents in most of the cells. The same type of asynapsis is found, for example, in *Zea* (BEADLE and MCCLINTOCK, 1928; BEADLE, 1930, 1932), *Crepis* (HOLLINGSHEAD, 1930; RICHARDSON, 1935), *Pisum* (KOLLER, 1938), *Secale* (PRAKKEN, 1943), *Godetia* (HÅKANSSON, 1945), and *Picea* (ANDERSSON, 1947). In these cases there is complete or nearly complete prophase pairing, the paired chromosomes subsequently separating. This separation takes place at different stages in different species. In *Godetia*, for example, HÅKANSSON

(1945) states that in a plant »lagen die I zu jeder Seite der Äquatorialplatte wie die Homologen von Bivalenten. Man bekam hierdurch den Eindruck, dass die Chiasmen erst spät aufgelöst werden, wenn die II schon gegen die Pole orientiert sind» (I and II indicate univalents and bivalents respectively). In *Alopecurus myosuroides* (with complete asynapsis), JOHNSSON (1944) found that the chromosomes were unpaired in late prophase (diplotene—diakinesis), and finally ANDERSSON (1947) points out that in spruce the chromosome pairs gradually fall apart at late pachytene and early diplotene. In *Ulmus* the pairing is more or less complete at early prophase and the number of bivalents per cell is observed to decrease from diplotene to I-M.

There seems to be a general agreement that the failure of chromosome pairing at I-M is probably due to the failure of real chiasma formation at prophase. The cause of this is, however, difficult to explain; PRAKKEN (1943) comes to the conclusion that »one may suppose that in some cases at least the decreased metaphase pairing depends upon physical changes in the chromosomes».

In this connection one should bear in mind the reluctance of bivalents to separate at the beginning of I-A, reported by LAMM in *Solanum* (1945), by ANDERSSON in spruce (1947), and observed in some of the cells in the present case (*Ulmus*). Such attenuated bivalents are also discernible in some of the figures in BEADLE's paper on *Zea* (Figs. 8 and 9; 1930), but are not discussed in the text. This phenomenon, too, may be ascribed to »physical changes in the chromosomes», or rather in the surrounding cytoplasm, though with the reverse effect, namely the marked tendency of paired chromosomes to remain together. It is reminiscent of the »stickiness» (cf. LAMM, 1945; OKSALA, 1945) described by BEADLE in *Zea* (1932), and chemically produced by ÖSTERGREN (1944) in root tips of *Allium*. According to BEADLE, the observed effects may be »due to a change in the physical properties of the protoplasm. The meiotic chromosomes have the appearance of being much more viscid in sticky chromosome plants than in normal plants». DARLINGTON and LA COUR (1940) consider that the cause of »stickiness» probably lies in a disturbed nucleic-acid metabolism of the chromosomes.

Disturbances of the normal time-relation between the pairing and division of chromosomes as another possible cause of reduced chiasma formation have been suggested by DARLINGTON (1937) in his precocity-theory of reduction division, and by SAX and SAX (1935) in their retardation-theory of reduction division (*cf.* also GUSTAFSSON, 1942). BARBER (1942), discussing experimental control of chromosome pairing in

Fritillaria, states, in connection with his reference to asynaptic genes as a cause of reduced chiasma frequency: »Presumably here the failure of chiasma formation is due to failure in the development of the torsion necessary for crossing over».

It has been established that chromosomes are built up by chains of protein molecules (MAZIA, 1941; FROLOVA, 1944; MIRSKY and RIS, 1947) and very likely consist of an inner coiled threadlike structure (»residual chromosome»; MIRSKY and RIS, 1947) and a surrounding mass or matrix. When crossing over occurs, these protein chains are broken off and reunited with another chain, in which process proteolytic enzymes may be involved. If this production of a certain enzyme is diminished or absent, no breakage is likely to occur and the chiasma formation is prevented. Many genetically controlled enzyme deficiencies are known (*cf.*, *e. g.*, HOROWITZ, BONNER, MITCHELL, TATUM and BEADLE, 1945). *But as long as the problem of chiasma formations remains unsolved all theories as to the nature of the phenomena which prevent this formation must necessarily be rather hypothetical.*

Attention should also be drawn to the well-known fact that the formation of chiasmata may be influenced by external conditions (see, *e. g.*, SAX, 1931; OEHLKERS, 1936; KOLLER, 1938; GOODSPEED and AVERY, 1939; GUSTAFSSON and NYGREN, 1946). In *Populus*, JOHNSSON (1940) found a varying number of univalents at I-M in 4 clones out of 16 investigated. He excluded hybridity and structural changes within the species as possible causes of the rather weak asynapsis, as well as genes controlling the failure of metaphase pairing. Instead he suggested that »the most probable cause is to be sought in external influences» bearing in mind that »PMC's of examined aspens were fixed from twigs which only a few days previously had been out of doors (in January and February) and transferred to a greenhouse», thus being exposed to great temperature changes when in a sensitive stage. Such temperature changes may occur in nature, too, at the time of reduction division in *Populus*, and also in elms (early spring), the result being disturbances of the meiosis. Now, the same method is used in obtaining flower-buds for fixation in elms, and these strong external influences may affect the behaviour of chromosomes at mitosis. However, seeds collected from elm No. 1 after free flowering gave rise exclusively to triploid plants (this elm is surrounded by normal elms), whereas plants originating from elm No. 2 (normal) had the diploid chromosome number. The crosses artificially performed in the greenhouse yielded the same result as regards the chromosome number of the progeny. However,

a few normal cells at least ought to be found if external influences alone were the cause of the asynapsis, not all PMC's being at the same stage simultaneously. That external influences are at work in *Ulmus*, too, will be seen from, *e. g.*, the varying number of bivalents per cell at I-M in different years (see Tables 2, 3 and 4). It should be noted that meiosis is more easily affected by external conditions in plants, where it is already in a state of unbalance, owing, *e. g.*, to the action of genes causing incomplete chromosome pairing.

SUMMARY.

(1) In *Ulmus glabra* HUDS. the somatic chromosomes were examined in diploids ($2n = 28$), triploids ($2n = 42$), and tetraploids ($2n = 56$).

(2) In 3 asynaptic elms ($2n = 28$) and 9 normal ones ($2n = 28$) meiosis was studied in PMC's and EMC's.

(3) In normal elms meiois was usually regular with 14 bivalents at metaphase I. The percentage of apparently good pollen grains varied between 75 and 100.

(4) The asynaptic elms showed more or less reduced chromosome pairing at prophase and metaphase I. In elm No. 1 a restitution nucleus was formed in about 79 per cent of the PMC's studied and a corresponding number of cells with dyads was formed (about 73 %). The other two asynaptic elms formed a two-nucleated interkinesis in 89 and 93 per cent, respectively.

(5) Crossings were performed between the different trees studied. In the cross asynaptic × asynaptic tetraploids as well as triploids were obtained. Asynaptic × normal yielded triploids only.

(6) The causes of asynapsis are discussed. The failure of chromosome pairing is probably due to the failure of real chiasma formation at prophase. The cause of this, again, has been discussed by many authors and is sought in »physical changes» in the chromosomes or in the surrounding cytoplasm; in disturbances of the normal time relations between the pairing and division of the chromosomes; in »asynaptic genes», preventing chiasma formation in some or other way; in genetically controlled enzyme deficiencies which exert an influence on the breakage and reunion of the chromatids.

(7) The influences of external conditions on the meiotic process are discussed on the basis of the different numbers of chiasmata per bivalent found in different years in the same tree.

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STUDIES ON VIVIPARY IN THE GENUS DESCHAMPSIA

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IN the genus *Deschampsia* there are in Scandinavia six species, which all have been determined with regard to their chromosome numbers (LÖVE and LÖVE, 1948, p. 25). Two species are diploid with $2n = 14$, *viz. D. atropurpurea* and *setacea*, and three are tetraploid, *viz. D. bottnica* and *flexuosa* with $2n = 28$ and *D. caespitosa* with the derived number $2n = 26$. The sixth species, *D. alpina*, is polyploid with chromosome numbers varying between $2n = 39$ and $2n = 56$. *D. alpina* grows in the *regio alpina* of the Scandinavian mountains and has hitherto been only known as viviparous. It has a western distribution, as the viviparous diaspores more easily take root in localities with high precipitation. It is known that the tetraploid *D. caespitosa*, in rare cases, may, an autumn or two, develop viviparous panicles; of the diploid *D. setacea*, HOLMBERG (1926 a, p. 181) has described a viviparous form.

FLOVIK observed a distinct difference in the degree of vivipary in three plants of *Deschampsia alpina* from Spitsbergen (1938, p. 284). One plant with the chromosome number $2n = 39$ had so weakly developed bulbils that they could hardly be detected without using a magnifying-glass, another with $2n = 41$ was less viviparous than a third with $2n = 49$.

Last year the writer obtained, from Dr. HARRY SMITH in Upsala, a form of *Deschampsia alpina* from the province of Herjedalen in the Middle of Sweden. It had the chromosome number $2n = 26$, was sexual and non-viviparous and gave seeds, from which some tens of seedlings could be raised. At the same time I was able to determine the chromosome number $2n = 52$ of several plants belonging to viviparous *D. alpina* grown in Northern Sweden and in Central and Northern Norway. From this it appears that the species *D. alpina* includes the polyploid series $2n = 26—39—52$. The basic number is consequently 13. In those cases where triploids with $2n = 39$ are able to develop some seeds, they give rise to plants with aberrant chromosome numbers. The triploids themselves are probably raised by the fusing of a reduced and an unreduced gamete.

A cytological investigation of two clones of *D. alpina* from Vassijaure, in the province of Torne Lappmark in northern Sweden, and from Knutshø, in the Dovre mountains in the middle of Norway, with the chromosome numbers $2n = 48$ and 52 respectively, made it clear that multivalents to a high percentage occurred in the first metaphase of the P.M.C's. Hexavalents as well as octovalents were at hand. This makes it rather probable that *D. alpina* is an autopolyploid species. Investigations of the embryology have shown that the development follows the normal type. Even in these high-chromosomal, viviparous forms just mentioned solitary embryo-sacs could be found that have a multicellular endosperm. Thus, maybe, tetraploids are sometimes able to develop some seeds.

Taxonomically, *D. alpina* is very closely allied to *D. caespitosa* (HOLMBERG, 1926 b, p. 167), and both species hold the same relation to each other as *Festuca ovina* L. to its viviparous sub-species, which some Scandinavian taxonomists rank as species (*cf.* GUSTAFSSON, 1946, p. 65). *D. alpina* with $2n = 26$ is a slenderly-growing type with short, thick leaves, which fits perfectly in the variation-series of *D. alpina*, but which — no doubt — could also be called a form of *D. caespitosa*. The triploid form mentioned by FLOVIK (*l. c.*) from Spitsbergen was also a slender, dwarfish type.

Studies in the meiosis of *D. caespitosa* have shown that, in the first metaphase of the P.M.C's, mainly bivalents are present, but that solitary tetravalents occur. Even if the species is to be looked upon as a tetraploid with a derived chromosome number, a stabilization of the formation of bivalents at meiosis has caused the species in nature to behave like a diploid. The original diploid *D. setacea* ($2n = 14$) is closely related to *D. caespitosa*. According to HOLMBERG (1926 b, p. 169), the Middle-European *D. media* (GOUAN) ROEM. et SCH., however, forms an obvious transition between these two species. It is the opinion of the author that the chromosome races of *D. alpina* are to be looked upon as polyploid derivatives of *D. caespitosa*. The cytological figures, especially the chromosome numbers, argue strongly in favour of such an interpretation (*cf.*, however, SCHOLANDER, 1934, p. 65). Concerning the connection of *D. caespitosa* to the diploids with $2n = 14$, nothing can be said at present.

From what precedes it will be seen that an investigation of the origin of vivipary in the species *D. alpina* should be based on a study of the manifold, allogamous species *D. caespitosa*. As early as in 1922 JENKIN succeeded in inducing vivipary in the last mentioned species

(p. 418). More important is, however, the discovery made by LAWRENCE in 1945 that originally non-viviparous Swedish and Finnish biotypes of *D. caespitosa* turned viviparous on cultivation in California. In this case the origin of vivipary must depend upon the environment. Of the external conditions which might contribute to the origin of vivipary in LAWRENCE's experiments, the light-factor is the most important. The investigations were worked out at Stanford University on the 37°30′ north latitude, but the material came from areas between the 55th and the 66th latitude. Consequently the step is at least 17 latitudes, which makes a considerable difference in the length of day between the original localities and the experimental field.

Fig. 1. Viviparous panicle of *Deschampsia caespitosa* raised after short day treatment.

In the summer of 1947 the present writer collected material of different biotypes of *D. caespitosa*, among other localities from the Dovre mountains in Central Norway and from Abisko in northern Sweden. The Dovre types were derived from the 62nd and the Abisko types from the 68th latitude. The plants hibernated in Upsala in the 60th latitude, and on the 5th of May, 1948, they were put into an isolation-cage to shut out the daylight. The plants were exposed to light for eight hours every day during two months between seven o'clock in the morning and three o'clock p.m. As a comparison, some viviparous and non-viviparous grasses were put into the cage; the results obtained are given in Table 1.

It is evident from the experiment, firstly, that only the northernmost biotype of *D. caespitosa* was affected in the direction of vivipary, secondly, that such forms as had an already fixed vivipary preserved this even after the treatment. In some of them, *viz. Festuca rubra* and *Poa stricta*, the short day seemed to effect an increase of the number

TABLE 1. *The effect of short daylight during eight hours on viviparous and non-viviparous grasses.*

Species	Normally viviparous	Normally non-viviparous	After treatment
Deschampsia caespitosa			
1. Abisko 68°	No	Yes	Viviparous
2. Dovre 62°	No	Yes	Non-viviparous
3. Upsala 60°	No	Yes	Non-viviparous
Deschampsia alpina			
1. Vassijaure 68°	Yes	No	Viviparous
2. Dovre 62°	Yes	No	Viviparous
*Festuca ovina *vivipara* 68°	Yes	No	Viviparous
*Festuca rubra *vivipara* 68°	Yes	No	Viviparous
*Poa arctica *depauperata* 62°	No	Yes	Non-viviparous
**elongata* 62°	No	Yes	Non-viviparous
Poa flexuosa 62°	No	Yes	Non-viviparous
Poa jemtlandica 63°	Yes	No	Viviparous
Poa herjedalica			
1. Javroaivve 69°	Yes	No	Viviparous
2. Helagsfjället 63°	Yes	No	Viviparous
Poa stricta 62°	Yes	No	Viviparous

of bulbils in the panicles. In all plants the development was delayed in comparison with the control-plants in normal daylight at the latitude of Upsala.

From the detailed investigations of TURESSON on *Festuca ovina* L. and its forms (1926—27, 1929—30, 1931) we know that, in the same species-complex, there will appear non-viviparous, semi-viviparous, and viviparous types. The vivipary is here genetically determined; it emerges in forms having different chromosome numbers, and these forms have the same ability to develop ecotypes as the non-viviparous ones. There is no reason to suspect the conditions to be different in the genus *Deschampsia*. In both genera there is an obvious tendency for the degree of vivipary to increase when there are higher chromosome numbers. *Festuca ovina *vivipara* apm. *tennforsiensis* ($2n = 42$) had the most distinct formation of bulbils in TURESSON's experiments (1930, p. 183), which agrees with the conditions found in tetraploid *D. alpina* ($2n = 52$). The origin of vivipary cannot, however, depend upon the chromosome number itself, as STÄHLIN has shown (1929, p. 350). It is only the degree of vivipary that is dependent upon the number. Interspecific hybridizations in the sense of ERNST (1918, pp. 500—512) as an explanation of the appearance of vivipary in *Deschampsia* are not likely,

whereas intraspecific ones are possible. Common to the experiments made by LAWRENCE and the writer is the finding that a change of the normal life-rhythm of the respective biotypes was sufficient to make them viviparous. The present writer presumes that in these cases the experimental plants of *Deschampsia* used are genetically and physiologically adapted to a fixed vegetation period and day-length. The Abisko plants, for instance, are adapted to a vegetation period between the beginning of June and the end of September, and to a resting period of about eight months. In the beginning of the vegetation period they get light for 24 hours in their natural environment. In the experiment at Upsala the plants had to begin their growing in the spring more than one month earlier than normal, besides which they were exposed to light only a third of the daily time to which they were adapted. Such a disturbance in the normal development of the plant brings about the effect in question, which appears from the investigations by HARDER of *Kalanchoe Blossfeldiana* (1948). A study in *Hyoscyamus niger* by LANG and MELCHERS (GREGORY, 1948, p. 89) revealed that this species needs 10 hours and 40 minutes' light every day during the growing-season to be able to develop flowers. If the plant was only exposed for 10 hours daily, it remained in a vegetative stage for 125 days. It has also been proved that the flowering depends on special hormones, the formation of which to a high degree depends on the length of daylight. By varying the light-factor in different experiments with *Kalanchoe*, HARDER was able to raise almost every transitional type between a well developed anthesis and a purely vegetative stage (*l. c.*, p. 119). SCHOLANDER has shown (1934, pp. 65—67) that in *Deschampsia alpina* the glumellae — that is, vegetative parts of the spikelets — are transformed into bulbil leaves. Hence, a formation of bulbils in *Deschampsia* is, on account of disturbances in the length of day, in perfect conformity with the experiments of HARDER. The successful demonstration of the fact that some substances must be at hand to render possible a formation of flowers in sexual plants throws new light upon the origin of various apomictic modes of propagation. Everything speaks in favour of different hormones and the moment of their action being genetically fixed and combined with a special life-rhythm. Disturbances of this normal rhythm — which may be of other kinds than in *Deschampsia* — will, depending on their degree, cause irregular meiosis, diplospory, apospory, or vivipary. By inter- or intra-specific hybridization between biotypes with such disturbances in the rhythm, different

apomictic types of propagation may originate and be stabilized by selection.

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FURTHER CYTOLOGICAL STUDIES ON ANURA AND URODELA

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INTRODUCTION.

AT the time when the present author published his first paper on the cytology of Urodela and Anura (WICKBOM, 1945), he was painfully aware of the fact that the solution of many of the problems concerning the evolution of the Anuran caryotypes demanded investigation of more species of primitive Anurans than were available to him. Because of the war, however, it was then impossible to obtain material from most foreign species.

During the last two years he has had the opportunity to complete the investigation with some species belonging to the suborder Opistocoela. The results are set forth here, and, in addition, further data on the cytology of some other Anura and Urodela are communicated.

MATERIAL AND METHODS.

The material used for the present study consisted of acetocarmin squash preparations of testes of the following species made according to the method described before (WICKBOM, 1945).

Anura.

Suborder Opistocoela.

Alytes obstetricans LAUR.	10 young males from Belgium. (For this material I am greatly indebted to Dr. J. PASTEELS, Bruxelles.)
	10 adult males from Belgium.
Bombina bombina L.	30 young males from the surroundings of the Weser, Germany.
Bombina variegata L.	10 adult males from Metz, France.
Discoglossus pictus OTTH.	10 adult males from Algeria and Sardinia.
Xenopus laevis DAUD.	10 young males from naturalists in Germany and Belgium.

Suborder Procoela.

Bufo regularis REUSS. 6 adult males from the Congo.

Urodela.

Suborder Salamandroidea.

Salamandra salamandra L. 10 adult males from Germany. They belong to the subspecies *taeniata* MERTENS.

Triturus alpestris LAUR. 10 adult males from Belgium. (For this material I am greatly indebted to Dr. J. PASTEELS, Bruxelles.)

10 adult males from the surroundings of the Weser, Germany.

All figures are drawn at a magnification of about 1500 ×.

RESULTS.

I. THE SUBORDER OPISTOCOELA.

The suborder Opistocoela contains, according to NOBLE (1931), the families Discoglossidae and Pipidae. They are considered to be the most primitive living Anurans except for *Ascaphus* and *Liopelma*.

A. *Alytes obstetricans*.

Alytes was formerly investigated by JANSSENS and WILLEMS (1909). Their observation, $2n = 32$, $n = 16$, has not been confirmed. *Alytes obstetricans* has $2n = 36$, $n = 18$ chromosomes. The set is composed of 4 pairs of long metacentric (WHITE, 1945) chromosomes; 6 pairs of middle size, Nos. 7 and 10 metacentric, the others acrocentric (WHITE, 1945); 8 pairs of small elements, all acrocentric (Fig. 1; Scheme 1). In very good preparations the members of the pairs 5, 6, 8, and 9 appear to be composed of two arms. The small arms, however, are less than 0,5 microns long and are heterochromatic. As these short arms do not develop chiasmata, the chromosome pairs 5, 6, 8, and 9 may be looked upon as functionally single armed, acrocentric elements.

In meiotic prophase, zygotene is characterized by the strong polarization also observed by JANSSENS and WILLEMS (1909). This is in agreement with other Anura (WICKBOM, 1945). In mid-diplotene, the chiasmata are easy to observe (Fig. 2). They are terminally localized and their number seldom deviates from one per arm pair. The cause

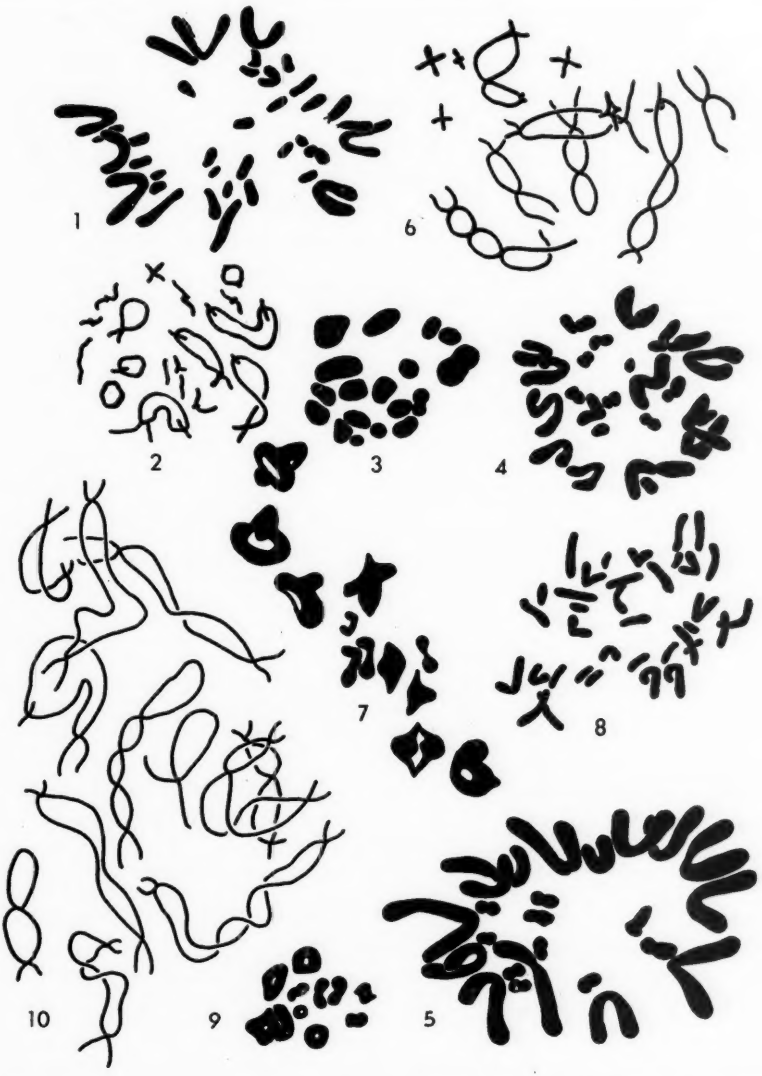
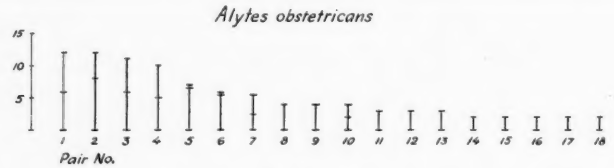
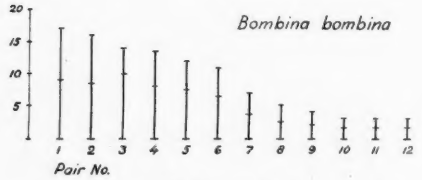


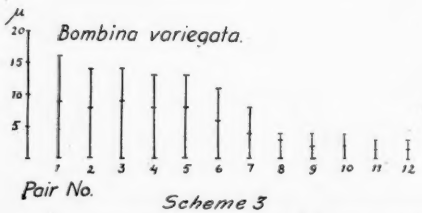
Fig. 1. Mitotic metaphase of *Alytes obstetricans*. — Fig. 2. Diplotene of *Alytes obstetricans*. — Fig. 3. I-M of *Alytes obstetricans*. — Fig. 4. Mitotic metaphase of *Bombina variegata*. — Fig. 5. Mitotic metaphase of *Bombina bombina*. — Fig. 6. Diplotene of *Bombina bombina*. — Fig. 7. I-M of *Bombina bombina*. — Fig. 8. Mitotic metaphase of *Xenopus laevis*. — Fig. 9. I-M of *Bufo regularis*. — Fig. 10. Diplotene of *Triturus alpestris*.



Scheme 1



Scheme 2



Scheme 3

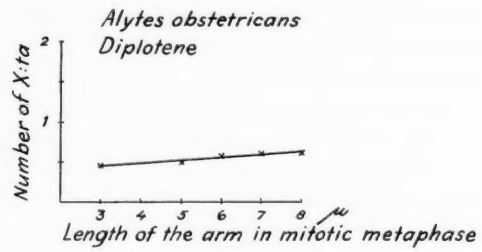
Scheme 1. The chromosome morphology at mitotic metaphase of *Alytes obstetricans*. — Scheme 2. The chromosome morphology at mitotic metaphase of *Bombina bombina*. — Scheme 3. The chromosome morphology at mitotic metaphase of *Bombina variegata*.

TABLE 1. *The relation between the number of chiasmata at diplotene and the length of chromosome arms at mitotic metaphase. Alytes obstetricans. 20 nuclei.*

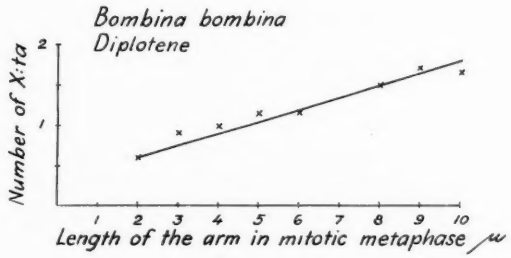
Number of X:ta	Length of arm in microns					Total
	3 and less	5	6	7	8	
2	—	4	7	2	2	15
1	265	72	72	18	18	445
0	15	4	1	—	—	20
Frequency	280	80	80	20	20	480
S (y)	265	80	86	22	22	475
$\bar{y}$	0,95	1,00	1,08	1,10	1,10	0,99 [1]

[1] 1,32 per bivalent.

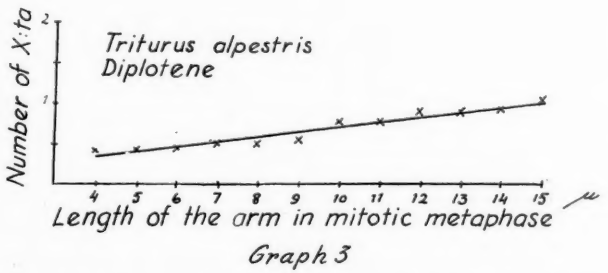
$Y = 0{,}04\ X + 0{,}84$. *Cf.* Graph 1.



Graph 1



Graph 2



Graph 3

Graph 1. The relation between chromosome arm length and number of chiasmata in *Alytes obstetricans*. — Graph 2. The relation between chromosome arm length and number of chiasmata in *Bombina bombina*. — Graph 3. The relation between chromosome arm length and number of chiasmata in *Triturus alpestris*.

of this localization and low frequency of chiasmata must be the terminal initiation of pairing, preproximal initiation of division and restricted time limit formerly described in Anura (WICKBOM, 1945). It is significant that the acrocentric pairs 5 and 6 have only 1,1—1,15 chiasmata per pair while the almost equal sized metacentric pair 7 has 1,65 chiasmata. The

same relation is observed comparing pairs 8 and 9 with pair 10. Although pairing must begin as early in the proximal as in the distal ends of the acrocentric chromosomes, the division evidently sets in so early at the proximal ends that there is not time for chiasma formation there. Thus, there is very low correlation between chromosome arm length and number of chiasmata in *Alytes* (Table 1, Graph 1), though it is a little higher than in other Anura (WICKBOM, 1945).

In diakinesis and I-M the chromosomes are highly contracted (Fig. 3) owing to the development of the major spiral, just as in most Anurans.

B. *The genus Bombina.*

Bombina bombina has not been the object of cytologic examinations before. *Bombina variegata* (*Bombinator pachypus*), however, was investigated by GALGANO (1933) who found $2n = 24$, $n = 12$. GALGANO reports nothing about the chromosome morphology except that 10 elements are very small. He also observed strong polarization at zygotene. The present author has confirmed these findings and can add that the caryotype resembles that of *B. bombina* (Fig. 4; Scheme 3).

Further, *Bombina orientalis* from China has been thoroughly investigated by SATO (1939) who also found $2n = 24$, $n = 12$. He reports 7 long and 5 short pairs, all with median or submedian centromeres.

GALGANO and SATO report long slender bivalents »of Urodelian type» (SATO, *op. cit.*) in I-M, *i. e.* minor spirals only from the *Bombina* species.

Bombina bombina also has $2n = 24$, $n = 12$ chromosomes. All elements are metacentric. Six pairs are long, one intermediate, and five short (Fig. 5; Scheme 2).

The frequency and distribution of chiasmata in *Bombina bombina* are given in Fig. 6; Table 2; Graph 2.

As will be seen, the number of chiasmata is correlated with length of chromosome arm. As compared with other Anurans the small chromosomes of *Bombina* have a lower, the long ones a higher number of chiasmata (*cf.* Table 2 and WICKBOM, 1945, Table 5, p. 271 ff.). The chiasmata are localized not only terminally as in other Anurans, but proximally as well in the long arms. Evidently the time limit is not so restricted as in other Anura. The long chromosomes get time to pair also in their proximal parts which must be near one another in the part of the nucleus being opposite to the polarized ends. For mechanical reasons this possibility to establish proximal contact points must be

TABLE 2. *The relation between the number of chiasmata at diplotene and the length of chromosome arms at mitotic metaphase. Bombina bombina. 20 nuclei.*

Number of X:ta	Length of arm in microns								Total
	2	3	4	5	6	8	9	10	
2	—	—	2	3	7	51	14	13	90
1	116	36	37	17	33	49	6	7	301
0	84	4	1	—	—	—	—	—	89
Frequency	200	40	40	20	40	100	20	20	480
S (y)	116	36	41	23	47	151	34	33	481
$\bar{y}$	0,58	0,90	1,03	1,15	1,18	1,51	1,70	1,65	1,00

$Y = 0{,}15\ X + 0{,}31$. *Cf.* Graph 2.

greater the longer the members of the pair — if the time limit is wide enough. There are never more than two chiasmata observed between a pair of chromosome arms of this species.

The same type of localization appears in *Bombina variegata* and can be traced from SATO's (1939) figures of *B. orientalis*. However, the number of chiasmata in *B. variegata* is significantly less than in *B. bombina* (0,9 per arm pair in *variegata*, 1,0 in *bombina*). The variation between different nuclei is greater in *variegata*, indicating a time limit factor. Evidently the time limit is more restricted in *variegata* than in *bombina*, which also appears from the fact that the small chromosomes in *variegata* seldom have more than one chiasma. In *bombina* some of them may have two chiasmata.

Concerning the spiralization in meiosis, the present author is able to confirm the above related observations of GALGANO and SATO (Fig. 7). The reader is referred to my previous paper for discussion on this point.

Three of the four known species of *Bombina* have now been cytologically investigated. The genus appears very uniform: *B. bombina*, *B. orientalis*, and *B. variegata* have 12 pairs of metacentric chromosomes. The Chinese species according to SATO (1939) has 7 large and 5 small pairs, the European both have 6 large, 1 intermediate, and 5 small pairs. The two European species are considered to have separated from one another quite recently in connection with the last glaciation period when the *Bombina* population was divided into one western (*variegata*) and one eastern (*bombina*) group (MERTENS, 1928).

Today, *variegata* is a highland and *bombina* a lowland form. However, they meet in some localities and hybridize (BATAILLON, 1910). The hybrids are fertile, at least to the second generation (HÉRON-ROYER, 1891).

Without cytologic investigations of the hybrid it is quite impossible to state anything with certainty about the chromosomal homologies between the two closely related species *Bombina bombina* and *B. variegata*, but the very similar caryotypes indicate that the differences are possibly not great.

C. *Discoglossus pictus.*

Discoglossus was investigated before by the present author (WICKBOM, 1945). The number reported then, $2n = 28$, was given with some reservation as the material was very limited and was restricted to embryonic mitoses only. Unfortunately, the author has had no opportunities to study the meiosis, but a number of good spermatogonial mitoses in the new material show without doubt that the number of chromosomes in *Discoglossus pictus* really is $2n = 28$. 10 elements are large and metacentric, 18 small. These small elements may be grouped into two fractions, one containing 8 acrocentric elements, and one containing 10 metacentric elements. A second little arm may, however, be seen in some of the »acrocentrics». It is, however, very small and heterochromatic. This peculiarity was not detected in embryonic mitoses where heterochromatin is not easily distinguished from euchromatin but it is very clear in the present material, especially after cold treatment. In the author's previous work (1945, Scheme 1, p. 252) the small arms of chromosome pairs 10, 11, 13, and 14 should be marked as heterochromatic and thus might not be looked upon as functional arms. In meiosis they possibly do not develop chiasmata.

D. *Xenopus laevis.*

The mitotic set of *Xenopus laevis* contains 36 chromosomes. 6 pairs are metacentric, 12 pairs acrocentric (Fig. 8). Thus the caryotype is the same as that of *Alytes*. The meiosis has been described before by the present writer (WICKBOM, 1945).

E. *Discussion.*

For comparative anatomical reasons the suborder Opistocoela is considered primitive (NOBLE, 1931). The cytological findings are in

agreement with this opinion. Deviations from other, more specialized Anurans are found regarding: (1) chromosome number and morphology; (2) chiasma frequency and distribution; (3) conditions of spiralization.

(1) In the three other suborders of Anura cytologically investigated the chromosome numbers are very constant, varying from 22 to 28. Of the Anomocoela, *Pelobates* has $2n = 26$; in Procoela, all Bufonids have $2n = 22$, all Hylids $2n = 24$; in Diplasiocoela, *Rana arvalis* has $2n = 24$ (or 26 in Italian forms, according to CEI, 1946), a great number of species $2n = 26$, while *Cacopoides* has $2n = 28$. See OGUMA and MAKINO (1937) and WICKBOM (1945) for the literature.

All chromosomes of all these Anurans are metacentric, the short arms of chromosomes with submedian or subterminal centromeres function in the formation of chiasmata.

In Opistocoela, the *Bombina* species with $2n = 24$ and all chromosomes metacentric correspond very well to the other Anura mentioned above. *Alytes*, *Xenopus*, and *Discoglossus*, however, deviate. The chromosome numbers 36 and 28 are the highest known among Anurans, and 12 chromosome pairs are acrocentric in *Alytes* and *Xenopus*, 4 in *Discoglossus*.

Similar conditions are met with in other groups of Vertebrata. In the most primitive suborder of Urodela, Cryptobranchoidea, the chromosome numbers of 14 species vary between 56 and 64 but were brought into correspondence with one another by the discovery that the number of arms is constant (MAKINO, 1935; SATO, 1936; MATTHEY, 1945; WICKBOM, 1945). The above mentioned authors assume that two rod-shaped elements may fuse to form one V, or *vice versa* (»ROBERTSON's law»).

In Reptilians, MATTHEY (1945 and earlier) has shown that the assumption that fusions according to »ROBERTSON's law» have occurred during the phylogeny allows the various chromosome numbers to be traced back to a common basic number.

Applying this »law» to *Alytes*' and *Xenopus*' 6 pairs of metacentric and 12 pairs of acrocentric elements, we find that they correspond to 12 pairs of metacentric elements, *i. e.* the condition found in the closely related genus *Bombina* and most other Anura. The same is true concerning *Discoglossus*' 10 metacentric and 4 acrocentric pairs.

This view is strengthened by the fact that *Alytes* has only 4 pairs of long chromosomes, while *Bombina* has 6 or 7 pairs. *Discoglossus* has 5 such pairs. Furthermore, the existence of heterochromatic regions around the centromeres of the metacentric chromosomes in

Urodela and Anura bears out the opinion that they have been formed by fusion of two acrocentric elements (WICKBOM, 1945).

It is, moreover, interesting that the number of chromosome arms, 48, found in Anurans is the »nombre fondamental» (MATTHEY, 1945) of Reptilia. Curiously enough, not only the same number of arms but also the same configurations as in *Alytes* and *Xenopus* (6 V, 12 I chromosome pairs) are found in *Sphenodon* (KEENAN, 1932).

There is a close resemblance between *Sphenodon* and the Triassic Rhynchocephalia, whence *Sphenodon* must be considered a very primitive Reptile, »a living fossil». Furthermore, the connection between the most primitive Permian Reptiles and the Stegocephalians is in reality so close that the delimitation of these groups from one another is quite arbitrary. It is generally accepted, too, that the Anurans have passed through a Stegocephalian stage, whence Anura and Reptilia are two branches of the same Tetrapod stock. Hence the occurrence of the same number of chromosome arms in Anurans and Reptilians, and the same configuration in primitive recent Anurans and the primitive Reptile *Sphenodon* is not so strange. Naturally the present writer does not believe that *Alytes* and *Xenopus* have developed from *Sphenodon* or *vice versa.* He only wants to set forth that, in all probability, Anura and Reptilia had a common origin and that the cytologic evolution of the two groups has run parallel. A series of fusions of acrocentrics to metacentrics is common for both groups. The fact that *Alytes*, *Xenopus*, and *Sphenodon* have arrived at the same point in this evolution (6 metacentric and 12 acrocentric pairs) is of no importance in this connection, only the method of evolution.

It seems highly probable that the chromosome number and morphology of *Alytes* and *Xenopus*, and, to a lesser degree, also of *Discoglossus* are relics of primitive conditions in Anura from which the present sets of most Anura were derived by a series of fusions of acrocentrics to metacentrics.

The difficulties with the centromeres in this connection are discussed by MATTHEY (1945) and WHITE (1945) and are by no means impossible to overcome.

Another point of importance in this connection is the great stability of Anuran chromosomes. In some genera, *e. g.*, *Bufo*, *Rana*, *Bombina*, and the family Hylidae all species have the same caryotype, often in spite of their enormous geographic distribution (*cf.* below). Only in the light of this fact it can be understood how ancient conditions may have been preserved in the suborder Opistocoela.

The number of chromosomes and chromosome arms in the most primitive suborder of Urodela, Cryptobranchoidea, do not, however, enable us to find any closer connection between them and the Anura—Reptilia branch. It is important to keep in mind that while approximately 48 arms in Anura and Reptilia seem to be a primitive feature, this number in Urodela is found only in the most specialized suborders. It may have developed from the 74—76 arms of Cryptobranchoidea by a series of unequal segmental interchanges and losses of small inert elements (MATTHEY, 1945; WICKBOM, 1945). As the caryotypes of the most primitive recent Anurans and Urodelians show so great differences, the occurrence of 24 metacentric chromosomes in the specialized salamanders says nothing about their relations to Anura and Reptilia. Primitive Urodela instead show a great resemblance to Dipnoi (WICKBOM, 1945).

(2) As regards chiasma frequency, *Xenopus* corresponds fairly well to most other Anura in having no correlation between chromosome arm length and number of chiasmata. The conditions in *Bombina* deviate, but may be brought into correspondence by the assumption that the time limit in *Bombina* is not so restricted as in other Anura. This must be considered as a primitive feature (WICKBOM, 1945, p. 285). *Alytes* may be looked upon as a transitional stage between *Bombina* and other Anura.

(3) Also the spiralization conditions in the meiosis of *Bombina* deviate from those found in other Anura. The occurrence of single spirals during meiosis is a characteristic feature in Dipnoi and Urodela (WICKBOM, 1945) and most Reptilia (MARGOT, 1946). However, it has been observed earlier in Anura by various authors.

The occurrence of single spirals in the meiotic chromosomes of Anura may be divided into the following groups:

(a) As a normal feature in females (GALGANO, 1937, 1941; DURYÉE, 1941).

(b) In intersexual germ cells, »cellule a sessualitá intermedia» (GALGANO, 1937, 1939, 1941).

(c) In abnormal cells in otherwise normal testes (GALGANO, 1933).

(d) As a normal feature in males of *Bombina* (GALGANO, 1933; SATO, 1939; WICKBOM, 1945, the present paper) and *Discoglossus* (CHAMPY, 1923).

It is difficult to determine whether the observation of JANSSENS and WILLEMS (1909) in *Alytes* belongs to group (b) or (c). In the

material of the present author, major spirals are invariably found in I-M.

It seems evident that the cause of the single spirals in groups (a) and (b) must be some physiological condition somehow connected with sex.

In the *Bombina* species, however, matters are different. The question is whether the single spirals are to be looked upon as a primitive feature, retained from remote ancestors, or as a secondary feature. It is, of course, impossible to give a decisive answer to that question. But the occurrence of single spirals among all primitive Tetrapod groups: Dipnoi (WICKBOM, 1945); Urodela (WICKBOM, 1945); Discoglossidae (the present paper); Gymnophiona (SESHACHAR, 1937); Reptilia (MARGOT, 1946) indicates a common origin of this feature, whence it may be assumed to be derived from ancestors common to these groups, possibly the Crossopterygians.

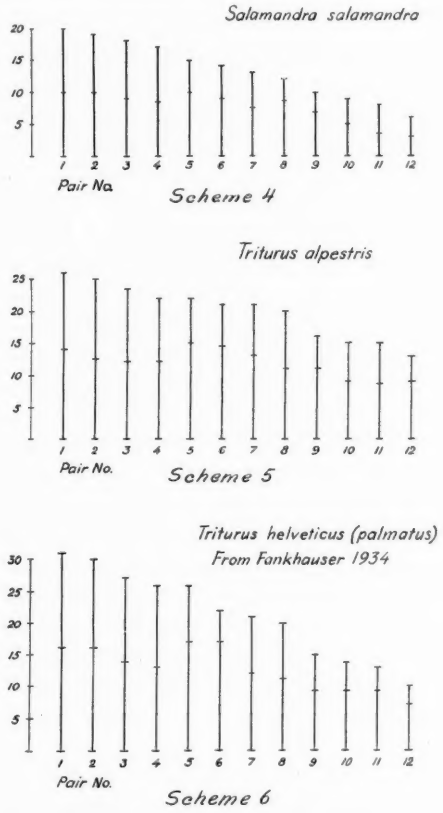
Thus several primitive cytological features are found in the suborder Opistocoela. It is, however, striking that not all of them are found together in every species. In *Bombina* we find single spirals and correlation between chromosome arm length and number of chiasmata; in *Alytes* and *Xenopus* acrocentric chromosomes and high chromosome number; in *Discoglossus* acrocentric chromosomes, relatively high chromosome number and single spirals. The occurrence of these primitive features in the cytology of Opistocoela may possibly be looked upon as a relic from very old ancestral conditions and it bears out the view that this is a primitive group.

II. BUFO REGULARIS.

Bufo is, aside from *Rana*, the Anuran genus with the largest number of cytologically investigated species. The chromosome number is reported in 11 *Bufo* species from Europe, Asia, North America, and South America (se OGUMA and MAKINO, 1937, for the literature). In this long list, however, there is no African species. Therefore it might be of some interest to contribute to our knowledge with the findings in *Bufo regularis*.

Unfortunately, the spermatogenesis in all specimens available had proceeded so far that no spermatogonial divisions were found. Therefore the investigation had to be confined to meiosis only.

The meiotic prophase in no respect deviates from that in other toads (WICKBOM, 1945). There are 6 large and 5 small chromosome pairs (Fig. 9), the chiasmata are distally localized and their number



Scheme 4. The chromosome morphology at mitotic metaphase of *Salamandra salamandra*. — Scheme 5. The chromosome morphology at mitotic metaphase of *Triturus alpestris*. — Scheme 6. The chromosome morphology at metaphase of a cleavage mitosis of *Triturus helveticus* (*palmatus*). From FANKHAUSER (1934).

is about one per arm pair. All chromosomes appear to be metacentric.

Thus the number of chromosomes in all *Bufo* species hitherto studied from all continents is $2n = 22$, $n = 11$. Six chromosome pairs are large, five small. All chromosomes are metacentric and the chiasma frequency equals one per arm pair. The genus *Bufo* thus appears very uniform in spite of its wide geographic distribution. This is a further evidence of the stability of Anuran chromosomes (*cf.* above).

III. SALAMANDRA SALAMANDRA TAENIATA.

The number of chromosomes has been described by many authors, first as early as 1882 (see OGUMA and MAKINO, 1937, for the literature). All investigators report $2n = 24$, $n = 12$.

The present author only wants to contribute the chromosome morphology, which appears from Scheme 4. The chromosomes are of common Salamandridae type, *i. e.* metacentric and very long.

From GALGANO's (1933) work it appears that the meiosis also follows the common Salamandridae type.

IV. TRITURUS ALPESTRIS.

The number of chromosomes in *Triturus alpestris* is reported by many authors to be $2n = 24$, $n = 12$ (see OGUMA and MAKINO, 1937, for the literature).

The chromosome morphology is demonstrated by Scheme 5. As will be seen by a comparison between Scheme 5 and Scheme 6, this chromosome set is very much like that of the closely related species *T. helveticus* (*palmatus*), as figured by FANKHAUSER (1934).

The meiosis of *T. alpestris* follows the normal Salamandridae scheme. The frequency and distribution of chiasmata appear in Table 3 and Fig. 10. The species resembles *T. vulgaris* in having few, localized chiasmata and therefore a restricted time limit. There is, however, a positive correlation between arm length and number of chiasmata (Graph 3).

TABLE 3. *The relation between the number of chiasmata at diplotene and the length of chromosome arms at mitotic metaphase. Triturus alpestris. 20 nuclei.*

Number of X:ta	Length of arms in microns												Total
	4	5	6	7	8	9	10	11	12	13	14	15	
3	—	—	—	—	—	—	—	2	1	1	1	1	6
2	—	—	—	—	—	3	4	8	23	10	11	6	65
1	14	14	29	30	15	57	11	35	36	19	18	8	286
0	1	1	1	—	—	—	—	—	—	—	—	—	3
Frequency	15	15	30	30	15	60	15	45	60	30	30	15	360
S (y)	14	14	29	30	15	63	19	57	85	42	43	23	434
$\bar{y}$	0,93	0,93	0,96	1,00	1,00	1,05	1,27	1,27	1,42	1,40	1,43	1,56	1,21

$Y = 0{,}06\ X + 0{,}61$. *Cf.* Graph 3.

SUMMARY.

(1) The number of chromosomes in *Alytes obstetricans* is 2n = 36, n = 18. Six pairs are metacentric, twelve acrocentric.

(2) The number of chromosomes in *Bombina bombina* and *B. variegata* is 2n = 24, n = 12. All chromosomes are metacentric. The individual chromosomes of the two species resemble each other very much, suggesting a close relationship.

(3) The number of chromosomes of *Discoglossus pictus* is 2n = 28. Ten pairs are metacentric, four acrocentric.

(4) The number of chromosomes of *Xenopus laevis* is 2n = 36, n = 18. The chromosome morphology resembles that of *Alytes*.

(5) In *Bombina* there is a positive correlation between chromosome arm length and number of chiasmata.

(6) In *Xenopus* there is no such correlation. *Alytes* represents a transitional stage between *Xenopus* and *Bombina*.

(7) In *Bombina* no major spiral is formed during meiosis.

(8) The following cytologic features found in Opistocoela are considered primitive: High chromosome number and acrocentric chromosomes; correlation between chromosome arm length and number of chiasmata; absence of major spirals during meiosis. These features must be considered as »relics» from conditions in remote ancestors of Anurans.

(9) The number of chromosome arms suggests a close connection between Anura and Reptilia. Primitive Urodela resemble Dipnoi in this respect.

(10) The number of chromosomes in *Bufo regularis* is n = 11. Six pairs are long, five short.

(11) The cytological uniformity of the genus *Bufo*, in spite of its world wide distribution, supports the view of the stability of Anuran chromosomes.

(12) The chromosome morphology of *Salamandra salamandra* is figured.

(13) The chromosome morphology of *Triturus alpestris* is figured.

(14) The meiosis of *T. alpestris* follows the common Salamandridae scheme, most closely resembling *T. vulgaris*.

(15) The mitotic chromosomes of the two Salamandridae species investigated, *Salamandra salamandra* and *Triturus alpestris*, are very long and metacentric, *i. e.* belong to the same type as other members of the family.

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MEIOSIS AND INTERSEXUALITY IN RECIPROCAL DREPANA HYBRIDS (LEP.)

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DURING my very detailed studies on the chromosomal conditions in the hybrids between *Pygaera pigra* and *curtula* as well as all their backcrosses, carried out during several years, I have been continually looking for other Lepidoptera species which could be subjected to similar studies in order to compare the conditions in them with the, in many respects peculiar, results obtained in the *Pygaera* crosses. The poverty of our Lepidoptera fauna made this task very difficult. It seemed, however, to be of importance, since one is easily tempted to generalize results obtained in regard to certain species which have been studied extensively.

In the summer of 1946 I finally succeeded in getting simultaneously the two closely related species *Drepana falcataria* L. and *Drepana curvatula* L. and in realizing the reciprocal crosses — named here *facu* and *cufa*. I had planned to carry out the backcrosses in the following summer, but the irregular emerging of the hybrids and the relative shortness of their life made this impossible. As a rule, several years are needed for an investigator to learn the differences in the developmental rhythm of the various hybrids and thus to be able to carry out the planned backcrosses. The sporadical results, obtained so far, seem to me, however, to be of such an interest that their publication is warranted.

EXPERIMENTAL RESULTS.

The two *Drepana* species, *curvatula* and *falcataria*, are relatively favourable for cytogenetic study, though naturally there are also difficulties present. Their flying periods coincide exactly. The pupae need therefore neither to be forced nor to be retarded in their development, processes that always cost much material. The libido is very strong, which as a rule is to be regarded as favourable for hybridization. In this case it is, however, so intensive that copulation takes place immediately after the moths have emerged and their wings have devel-

oped. Certain precautions must therefore be taken. In order to prevent undesirable copulation and to secure the virginity of the females, the sexes must be separated already at the pupal stage. The moths emerge early in the morning from the pupae, and copulation takes place, as mentioned, immediately after the wings have hardened. It usually lasts some hours, but may be done in a few minutes. It is therefore not necessary to watch the whole night in order to isolate the copulated pairs, as when working with Sphingids.

The females lay their eggs in the following night. The eggs of *curvatula* are laid in a long straight row. I have counted over 20 eggs in one such row, the batches, however, usually containing 8—12. The *falcataria* female also lays its eggs in rows, a group containing, however, rarely more than 3—4 eggs. *Falcataria* also lays single eggs, whereas this is not the case with *curvatula*.

The first F_1 cultures of *cufa* and *facu* both gave 100 % of caterpillars, whereas in the pure cultures of *curvatula* and *falcataria* only 90 % caterpillars developed, a fact evidently caused by lethal factors. (*Cf.* Table 1.)

TABLE 1.

	Not developed	Half developed	Adult caterpillars	Number of hatched caterpillars	Percentage of hatched caterpillars	Total of eggs
4672 *falcataria*	—	4	6	150	93,3	160
4774 »	57	—	18	159	67,9	234
4674 *curvatula*	1	—	2	104	97,2	107
4775 »	50	12	95	123	43,9	280
4776 »	?	?	?	?	?	321
4777 »	1	—	95	82	46,1	178
4675 *facu* F_1	—	—	—	183	100	183
4770 » »	22	4	51	173	69,2	250
4676 *cufa* F_1	—	—	—	200	100	200
46124 *facu* F_2	36	—	3	—	—	39
46125 » »	73	—	—	—	—	73
46127 » »	42	—	1	—	—	43
46128 » »	185	11	24	—	—	220
46129 » »	208	3	3	—	—	214
46130 » »	175	2	2	—	—	179
46132 » »	141	1	1	1	0,7	144
46134 » »	67	27	9	1	1,0	104
4764 *falcat.* × *cufa*	168	9	7	5	2,7	189

The development of the larvae is very rapid — it is accomplished in about three weeks, in the hybrids even faster — and meiosis in the

testis already begins at the last but one larval stage. Occasional meiotic divisions may, however, still be found in the fresh pupae. The earliness of spermatogenesis is very favourable for cytological studies, as most larval diseases — genotypic as well as extrinsic — are most disastrous at the last larval stages. In the species with early spermatogenesis material of it may as a rule be secured, even if the culture is destroyed later. This is, however, not the case when spermatogenesis takes place so late that meiosis occurs at the pupal stage.

The greatest difficulty in the not very numerous cultures of the hybrids and the pure species is in my opinion the extreme irregularity of the development of these moths. As a rule, both *curvatula* and *falcataria* develop a not insignificant number of moths without hibernation. They are thus partially bivoltinistic. According to the investigators of the silkworm, bivoltinism is caused by a recessive gene which, however, is very dependable on extrinsic factors, especially temperature. In warm summers usually a greater percentage of the moths develop without hibernation, in colder summers again a smaller. In the summer of 1945 I accordingly obtained in a *curvatula* culture 9 ♂♂ and 1 ♀ without hibernation, in another culture of the same size only 1 ♂ and 1 ♀. In the summer of 1946 12 ♂♂ emerged already during the summer, in the following spring 13 ♂♂ and 14 ♀♀. In the unusually warm summer of 1947 there developed in three individual-rich cultures only 2 ♂♂. Of *falcataria* I have had a smaller material which gave no bivoltinistic moths.

The hybrids of the Lepidoptera usually show a much more rapid development than the pure parental species. In many hybrid cultures not a single pupa hibernated, although the parents as a rule are not bivoltinistic, which fact renders backcrossing impossible. Of the hybrids now in question the *cufa* culture of 10—14. VIII, 1946 developed no fewer than 26 ♂♂, and in June, 1947, only one single ♂. The female pupae of this culture do not develop. They were still alive in the summer of 1948, but showed no sign of development. This depends obviously on a sublethal combination of the sex chromosomes. The *Y* chromosome of *curvatula* is incompatible with the *X* chromosome of *falcataria*. This case seems to be analogous to *Deilephila elpenor* ♀ × *Metopsilus porcellus* ♂ (FEDERLEY, 1929, 1932). STANDFUSS, however, claims to have obtained a few females of the hybrid *cufa*. Perhaps the behaviour of the different geographic races diverges in this respect.

The reciprocal hybrid *facu* showed a different behaviour in the summer of 1946. The pupae 11—19. VIII gave 46 ♂♂ and 17—24. VIII

59 ♀♀. 19 pupae were still alive after the hibernation. They were all ♂♂, and 10 of them developed into moths. 9 were still alive in July, dying, however, in the course of this month.

After these experiences in the summer of 1946 I had hoped to be able to carry out backcrosses in the following summer, especially with the *facu* ♀♀. This was prevented by the fact that I obtained only 2 *curvatula* ♂♂, and that there emerged to 36 *facu* ♂♂ only a single female, and even this not until the 12th of September, when no males were present any more. The life duration of the *Drepana* moths is very short. After four days they are dead, and I have been able to bring about copulation only between individuals of 2—3 days.

The two *facu* cultures thus behaved quite differently in regard to the emergence of the females. I hope to be able in the summer of 1948 to carry out the planned backcrosses with the *facu* females and the males of the two parental species, which would be of great interest in regard to the chromosomal conditions as well as sexuality.

In the summer of 1946 12 copulations were obtained between *facu* moths *inter se*. Since the *facu* culture had given 100 % caterpillars, I hoped to get a great number of F_2 individuals. (The chromosomal conditions were still unknown to me in August, 1946.) The results of these crosses are seen in Table 1. 4 of the 12 females did not lay any eggs, the 8 remaining gave two caterpillars in all, which developed regularly. The gonads of the latter were fixed, both turning out to be intersexes.

In the summer of 1947 I succeeded in obtaining a backcross between a *cufa* ♂ and a *falcataria* ♀. Of 189 eggs only 5 (2,7 %) caterpillars hatched (*cf.* Table 1). Two of the caterpillars died immediately after the hatching and one was killed through an accident. The two remaining developed well and gave moths which externally were pure males, being only slightly lighter in colour than the brown *curvatula* ♂♂.

The two parental species differ mainly as to their colour. *Falcataria* has a white-yellowish ground colour, whereas *curvatula* is brown with a violet hue. *Falcataria* has two dark grey patches in the middle of the fore-wing, the marginal one of which is especially large, *curvatula* again having two quite small patches.

The hybrids may be briefly characterized as having the brown colour of *curvatula* but lighter, whereas the pattern is from *falcataria*. The large dark patch of *falcataria* appears distinct and unimpaired. In appearance the *cufa* and *facu* males are identical. The two backcross males *falcataria* ♀ × *cufa* ♂ are, as mentioned, scarcely lighter than the F_1 individuals.

The laying of eggs is intermediate. The eggs are laid in rows, which, however, are never so long as the ones of *curvatula.*

CHROMOSOMAL CONDITIONS.

The parental species both have 31 chromosomes, a haploid number commonly occurring in Lepidoptera. I have not been able to detect any difference in the chromosome complements of the two species. As is usual in moths, the chromosomes are small and uncharacteristic in shape

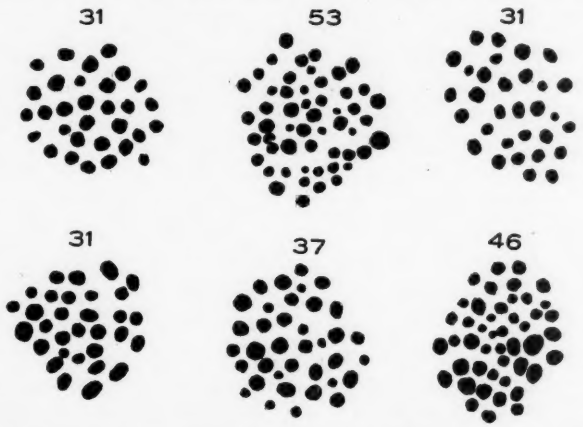

Fig. 1. First spermatocytes. In the upper row to the left *curvatula,* to the right *falcataria*; the four other groups with 31, 37, 46 and 53 chromosomes *cufa*

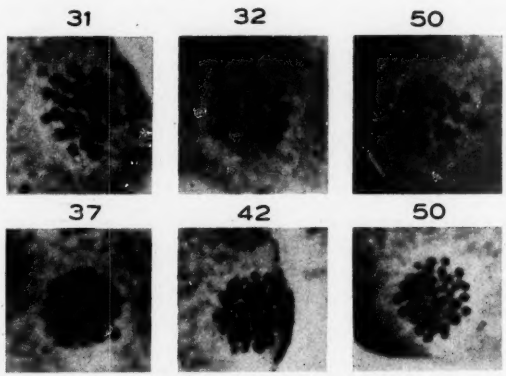

Fig. 2. First spermatocytes. In the upper row to the left *falcataria* with 31 and *facu* with 32 and 50 chromosomes. In the lower row *cufa* with 37, 42 and 50 chromosomes. Many chromosomes not conspicuous owing to irregular equatorial plates.

(*cf.* Figs. 1 and 2). The chromosomes in the oocytes are, as always, somewhat larger and more elongated than in the spermatocytes (*cf.* Fig. 1 with 3, and 2 with 4).

Gametogenesis in the hybrids is in so far interesting as it is quite analogous to that observed in the *Pygaera* hybrids. In spermatogenesis

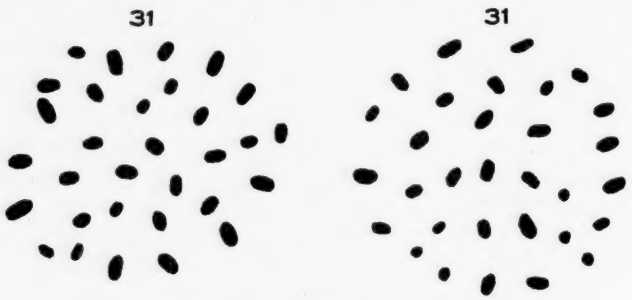

Fig. 3. First oocyte of *facu* with 31 chromosomes.

we find a weak affinity and loose pairing between the chromosomes of the different species (Figs. 1 and 2), whereas pairing is quite normal in oogenesis. In 7 *facu* hybrid females I have in numerous eggs counted

Fig. 4. First oocyte of *facu* in three photographs at different level.

the exact number of 31 chromosomes, and the plates have a regular structure. The chromosomes of *curvatula* and *falcataria* accordingly pair regularly with each other.

Spermatogenesis is rather similar in *cufa* and *facu.* In both, beautiful premeiotic stages are to be found. Synizesis is often observed, pachytene is as a rule well-developed, and diakinesis is also quite distinct, often already with univalents. The equatorial plates, however, are unfortunately not very good, contrary to the usual state of affairs in Lepidoptera. This holds true, however, even of the pure species, and

TABLE 2.

		Chromosome numbers																													
		31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	n	M	δ	m
cufa				1	1																						2	33,5		
		3	3	10	5	7	6	8	3		2																47	34,9	± 2,24	± 0,33
					1		1																				2	35,0		
			1	1	3	1	4	4	1	1			1	2			1										20	37,3	± 3,59	± 0,80
									1	1	2			2	2	1											9	41,8		
										1	3	2		1	1	2											10	41,8		
						1	1	4	1	2	4	3	6	1	2	3	2	3	1		1	1					35	41,9	± 3,61	± 0,61
						1	1								1	2	1		1								7	42,7		
								1		2	1	1		3	2	3	2	4			1	1					21	44,2	± 3,51	± 0,72
											1										1						2	45,0		
									1						1						3						5	46,4		
																1	1		1		1						4	47,0		
															1	1		1	1	1				1			6	47,7		
		3	4	12	10	10	13	17	7	7	13	6	7	9	10	13	7	8	4	1	7	1		1			170			

Number of cells

TABLE 3.

		Chromosome numbers																													
		31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	n	M	δ	m
facu							1	1					1	1													4	39,5		
								1	1		1	1			1												5	40,0		
			1				1	1	1	3	4	1	2	1		1		2			1					19	40,9	± 3,9	± 0,89
										1	1	2	1	3		3	2	1	1							15	43,6	± 2.6	± 0,67
										1	1			3		1	1	1	2							10	44,2		
											1	1			1	2	2				2		1				10	45,9		
											1		1		2	2		2			1	1	1			1	12	46,8		
			1				2	3	2	5	9	5	5	8	4	9	5	6	3		4	1	2			1	75			

Number of cells

	54	55	56	57	58	59	60	n	M
facu F_2	1		1	2		1	1	6	57,2

only after a long search is a plate found where all the chromosomes are at the same level. Consequently the plates do not give good photographs, as is to be seen from Fig. 2, and only a relatively small number of plates are to be found in which the chromosomes can be counted exactly.

The irregularity of the pairing conditions in the spermatogenesis of the reciprocal hybrids is best seen in Tables 2 and 3. An examination of Table 2, representing the chromosomal conditions in *cufa*, reveals the chromosome numbers 31—53, *i. e.* all numbers from normal pairing to

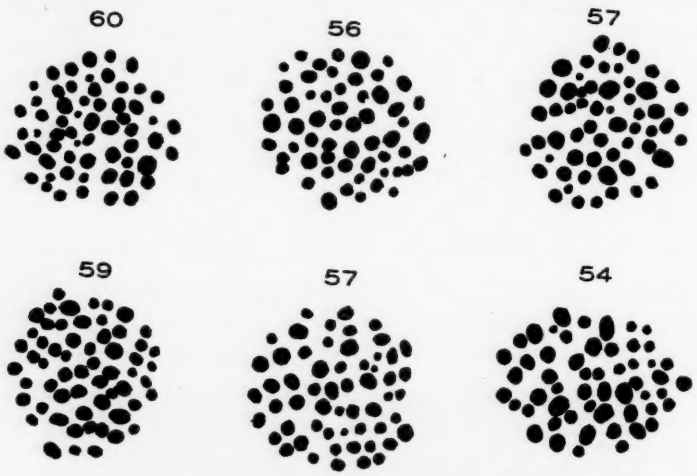

Fig. 5. First spermatocytes of *facu* F_2.

only 9 pairs. It is astonishing how differently the different individuals behave. In one the limits lie at 31 and 40, in another at 44 and 53. One shows a relatively narrow range of variation from 31 to 40, while in another this is much wider: 35—51. The mean numbers in 13 testes vary from 33,5 to 47,7. From the table we finally see that the fertility of the hybrid males must be very low, a fact borne out also by the single successful backcross. (*Cf.* Table 1.)

In *facu* (Table 3) we find a corresponding situation. The extreme numbers are here 32 and 55. The number of plates examined of this species is much smaller. The distribution is, however, also wide, varying, as in *cufa*, greatly between different individuals. In *facu* the mean numbers lie between 39,5 and 46,8, showing thus a smaller range of variation than *cufa*, though this may depend on the much smaller material.

The second division does not differ much from the first. The size of the chromosomes is naturally smaller, since they are univalent. This renders their examination more difficult. The plates are often irregular, and in many cases it may be observed that a few chromosomes are retarded in the division, not being included in the plates, which is a feature commonly found in hybrids of Lepidoptera. Whether this depends on inversions or other irregularities at chromosome pairing, I have not been able to decide here or in the other hybrids.

I succeeded in obtaining only one F_2 individual in which spermatogenesis could be studied. Still, it is possible that we here have a transformed female (*cf.* p. 61). In any case a few good plates could be found. The chromosome number varies in these between 54 and 60. Fig. 5 illustrates these plates. Attention is drawn to the remarkable size of the chromosomes, from which it may be concluded that a considerable number of them are bivalent, and that it is the sperms with the diploid — or almost diploid — chromosome number which give rise to the offspring.

INTERSEXUALITY.

Since GOLDSCHMIDT analysed his famous *Lymantria* intersexes and founded his theories concerning sex on these analyses every investigator working with Lepidoptera has quite naturally looked forward with interest to seeing how hybrids between other Lepidoptera species will behave in this respect. SEILER found in his triploid *Solenobia* hybrids other intersexual types than GOLDSCHMIDT in *Lymantria*. In my *Pygaera* crosses, which I carried out in repeated backcrosses in different directions, I found among tens of thousands of individuals only two intersexes and a few gynandromorphous individuals. This case obviously differs from the *Lymantria* intersexes of GOLDSCHMIDT, being also more difficult to analyse owing to the complicated chromosomal conditions. I was therefore interested in seeing how the *Drepana* hybrids would behave in regard to sexuality.

In the summer of 1946 I fixed, unfortunately, almost exclusively testes of the young caterpillars, since the very young ovaries are of little interest for chromosome studies. I was not aware that these very young stages would be especially interesting in regard to the beginning sex reversal. 5 gonad pairs were fixed in the summer of 1947, after I had then examined two intersexes of the F_2 generation of *facu*, and the gonads of the caterpillars had shown signs of a morphologic transformation. I did not want to sacrifice a greater number of caterpillars,

as I hoped to be able to carry out a number of backcrosses. This was, however, unrealizable, as no moths of the pure species emerged at the same time. The material is therefore very restricted, showing, however, many features which considerably resemble the *Lymantria* intersexes.

In the single *cufa* culture and the backcross of this hybrid with a *falcataria* ♀ no trace of intersexuality could be observed. All the intersexes have been found in the hybrid *facu*, in both the F_1 and F_2 generations. In the F_1 culture 4675 there occurred, peculiarly enough, no intersexes, although a considerable number of caterpillars were dissected. It is true that mainly testes were searched for in order to obtain material for the study of spermatogenesis. The relative number of the sexes, *viz.* 65 ♂♂ and 59 ♀♀, all morphologically either pure males or females, indicates too that no reversal has occurred. The four pairs of fixed testes and the seven ovaries of imagos did not, either, reveal any trace of intersexuality. The *facu* culture 4770 again contained among the five fixed caterpillars four intersexes and only one pure male. The latter was fixed already on the 5th of July, the intersexes again on the 19—24th of July.

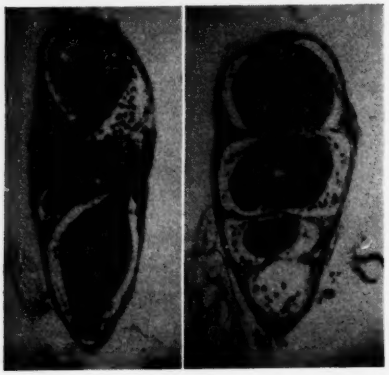

Fig. 6. Young ovary of *facu* F_1. The dark basal parts consist of young oocytes, the light parts of young spermatocytes, some of which have already formed characteristic cysts, which resemble blastulae.

The four intersexual gonads exhibited different stages in the transformation of ovaries into testes. They are described below.

Gonad I. — The first caterpillar killed was adult and possessed two gonads, which I described as »ovaries?» at the fixation. They were still very small, but were more rounded than typical ovaries.

The consecutive series of sections revealed a gonad with four equally developed follicles. In the basal part they are typical ovaries in which the differentiation of the cell elements has not yet begun or is just beginning. Distinctly differentiated egg and nursing cells cannot be discerned as yet. The ovarial tissue is clearly limited and is much more intensely stained than the bordering transformed testis tissue, as is seen in the gonad at the left in Fig. 6. The intervening tissue contains, as seems usually to be the case, numerous degenerating, strongly pycnotic

cells. The part being transformed consists of gonia which are packed together and represent an indifferent type. In the apical part of the uppermost follicle a typical cytocyst of the characteristic blastula type with a large cavity is seen, however. Quite normal spermatogonial mitoses which lie tightly packed as in a pure testis are also to be found. Phagocytes are very common, as is clearly seen from the photographs. To the right in the section of the same gonad a pure testis follicle is situated above, in the following follicle the section has cut at the left a part of the ovarial tissue. Morphologically, the section closely resembles a young testis.

The partner gonad is very similar to the one just described, showing the same degree of transformation.

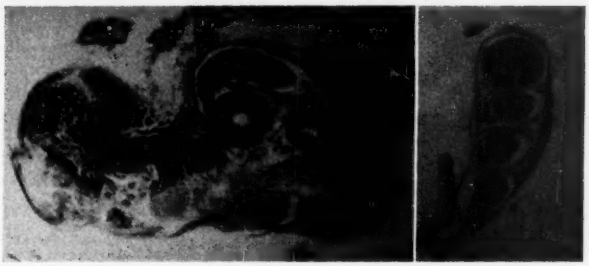

Fig. 7. To the left a somewhat older ovary of *facu* F_1. Differentiation into egg and nursing cells is already distinct. The gonia of the follicle to the left transformed into spermatogonia. To the right the still undeveloped partner gonad, intermediate between ovary and testis with indifferent cells.

Gonad II. — Fixed on the 24th of August. One of the gonads is considerably larger than gonads I. The follicles have already a winding appearance. The differentiation into egg and nursing cells has clearly begun in the basal part, as is distinctly seen in Fig. 7. The transformation is going on in the apical part; the gonia are, however, mainly indifferent and closely packed, revealing the characteristics neither of oogenesis nor of spermatogenesis. Gonocysts or cytocysts typical of spermatogenesis do not occur as yet.

The partner gonads are in this case of somewhat different type. One is still very small, and consequently the follicles are as yet not winding. The differentiation of the young oogonia has just begun, indicating only faintly a transformation, as seen in the rounded follicle in Fig. 7 to the right.

The four follicles in the two gonads are *inter se* fairly similar.

Gonad III. — Fixed on the 22nd of August. The two gonads show rather different degrees of development.

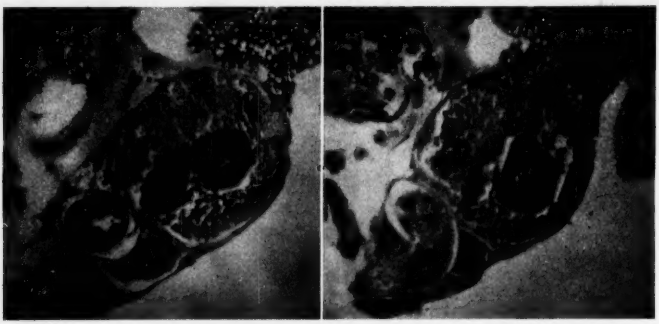

Fig. 8. An older gonad of *facu* F_1 with darker ovarial tissue in the middle of the large follicle, otherwise consisting mainly of indifferent gonia.

The larger and more developed of the gonads is of considerably greater size than those described above. The follicles are long and consequently winding. In the basal part they are still pure ovaries with well-differentiated egg and nursing cells. In the apical part the transformation has just begun. It is considerably enlarged and contains mainly indifferent gonia, but typical spermatocyte meiosis may also be discerned sporadically. A peculiar feature is that they do not lie in typical cytocysts but are distributed amongst the gonia. Typical gonocysts with numerous mitoses are also found. This case is exceptional in one respect. The ovarial part occupies the middle of the follicle while the periphery consists of indifferent cells, as clearly illustrated by Figs. 8 and 9. The distinctly limited ovarial part is situated

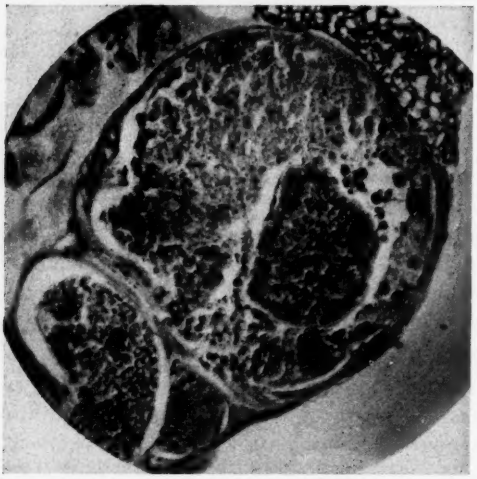

Fig. 9. The gonad of Fig. 8 to the right in higher magnification.

in the centre of the large follicle. Degenerating and pycnotic cells are also found.

As regards its ovarial part the smaller partner gonad is similar to the one described above, though the transformation has proceeded less far. The periphery of the apical part of the somewhat enlarged follicle contains only indifferent gonia and degenerating cells. Typical testis elements are not discernible as yet, contrary to the condition in the larger gonad.

The two gonads also contain a great number of phagocytes.

Gonad IV. — Fixed on the 22nd of August. The two gonads are quite dissimilar.

The one is *a normal testis* with a quite regular course of spermatogenesis. The ducts, too, have the character of vasa deferentia. All the four follicles have a typical Verson's cell and reveal all stages of spermatogenesis.

The other gonad, which obviously must also be regarded as a testis, since it contains all spermatogonial stages from gonia to ripe sperms, has in addition in the basal part of all the four follicles a distinct ovarial tissue with indubitable egg cells, as illustrated by Figs. 10 and 11. Especially the typical, long and strongly developed oviduct must be taken into consideration. It is an unmistakable indication of the abnormality of the testis. The distinct boundary between the ovarial and the testicular part is clearly illustrated in Fig. 10, and also in the more strongly magnified Fig. 11. The ovarial tissue and especially the thick epithelium of the oviducts stain much more intensely than the testis elements. All the follicles show about the same degree of transformation, containing also a Verson's cell.

Phagocytes are here scarce; obviously they have already fulfilled their mission.

One of the two F_2 *individuals* was killed already at a larval stage, the other as a pupa immediately after its sex had been established and its gonads were fixed. Microscopical examination showed both of them to be intersexes, although of quite different type.

The pupal gonad — the originally separated gonads had here fused — exhibited in two follicles a normal course of spermatogenesis. Although in the pure species as well as in the F_1 individuals meiotic divisions abound already before the last moult in the caterpillar, being scarce in the pupa, the F_2 pupa now in question contained all stages of spermatogenesis, especially younger ones, though in only two follicles. The remaining six follicles were too young, which I have interpreted

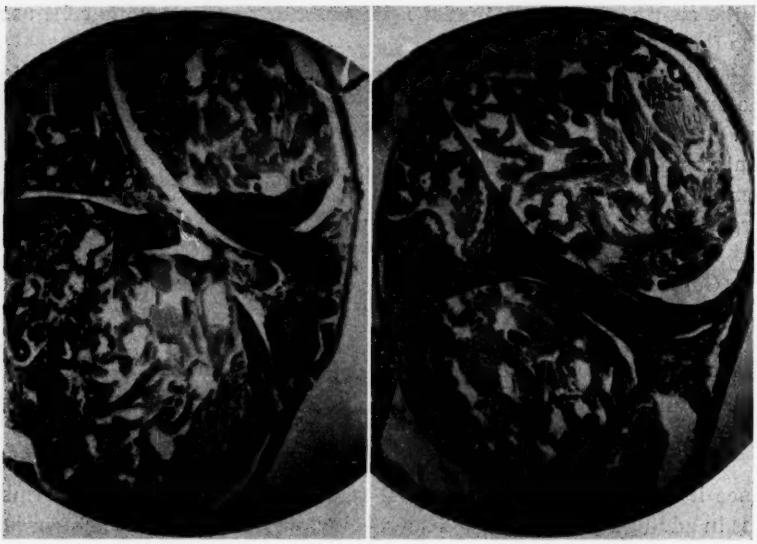

Fig. 10. Ovary transformed into testis in a *facu* F_1 individual. The dark basal regions still contain typical egg cells, in the other parts spermatogenesis is going on, showing all stages up to ripe sperms.

Fig. 11. Basal part of a follicle in Fig. 10 more strongly magnified with distinct egg cells.

as indicating that we here have a transformed ovary. Spermatogenesis is quite normal in the two follicles. All stages are present, and the ripe sperm bundles often contain externally normal sperms. As always, numerous bundles with apyrene sperms are also to be seen, and even the eupyrene ones often have an abnormal appearance. In four follicles a typical Verson's cell is found.

The larval gonads represent a completely different type. Even at the time the caterpillar was dissected they gave to a certain extent the impression of young testes. This is especially true of one of the gonads, whereas the other had a knoblike outgrowth, which is never found in a young ovary.

The gonad which more resembled an ovary proved, when sectioned,

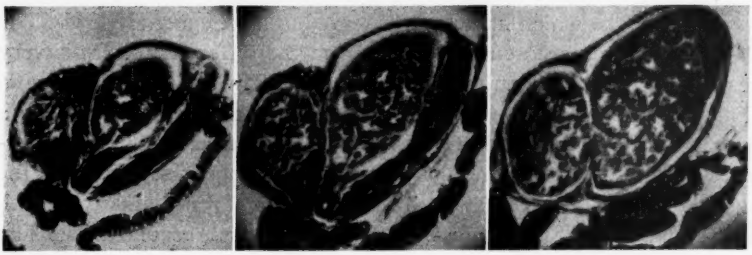

Fig. 12. Gonad of a *facu* F_2 individual. Photograph of three sections of one gonad. The two follicles to the right are almost pure ovarial follicles, showing incipient pycnosis. The two follicles to the left with ovarial basal parts, the apical parts transformed into testes. Typical cytocysts, resembling blastulae (the photograph to the right).

to be a young ovary in which the differentiation of the various cell elements had not yet begun. Most cells are at synizesis—synapsis or pachytene stage. Those which lie nearest to the ducts are partly degenerated and pycnotic, which perhaps indicates a beginning transformation. The only feature which can be interpreted as a sign of intersexuality is the enlargement of the apical end of one of the gonads. The cells situated in this part are indifferent gonia.

The gonad resembling a testis is of quite another type. It consists of two typical ovarial follicles, which are similar to the partner gonad described above, and of two others which must be characterized as testis follicles. Spermatogenesis in these has reached only the first stages. Most cells represent young growing spermatocytes, and the oldest form the cytocysts with a large cavity. As usual, many gonocysts are situated in the apical part and in several places typical centres of

gonial mitoses may be observed, which are never found in the ovaries. A Verson's cell is not present in the entire series of sections. As illustrated by Fig. 12, the transformation of the large follicle is almost completed, no ovarial cells being found any more. The follicle to the left in the section in its turn clearly exhibits, in its centre, the intersexual character in that the basal part still shows distinct ovarial elements and is sharply limited against the testicular part.

DISCUSSION.

The results obtained when *Drepana curvatula* and *falcataria* are crossed are in many respects of general interest.

Firstly, we find here in the F_1 hybrids the same peculiar *chromosomal conditions* which have been observed in the *Pygaera* hybrids. The affinity of the chromosomes derived from the different species is very weak in the spermatogenesis of the reciprocal hybrids *facu* and *cufa*, being in addition highly variable. This results in the pairing of only a few chromosomes, which in turn gives rise to gametes containing different combinations of *curvatula* and *falcataria* chromosomes. In the oogenesis of the *facu* female all the 31 *falcataria* chromosomes pair with the 31 *curvatula* chromosomes, according to which regular Mendelian ratios should be expected if all the combinations of the *cu-* and *fa-*chromosomes are viable. The question how this different reaction of the combined *cu-* and *fa-*chromosomes in oogenesis and spermatogenesis can be explained must be subjected to further study. The fact that similar phenomena have been observed in the *Drepana* crosses as in the *Pygaera* hybrids seems to speak in favour of the opinion that the conditions in the oogenesis are more favourable for a normal conjugation of the chromosomes than those prevailing in the spermatogenesis. I hope that the backcrosses of the *facu* females with the males of the pure species will throw more light on this problem.

The females of the cross *cufa* are obviously, at least in the combination in which I have obtained them, sublethal, as not one of the numerous pupae gave an imago. Here we have a parallel phenomenon to the behaviour of the female pupae in the Sphingid hybrids which live several years, being, however, unable to complete their metamorphosis (FEDERLEY, 1929, 1932).

The *facu* culture 4770, again, is in regard to sexuality analogous to the famous *intersexuality cases* in *Lymantria dispar* studied by GOLDSCHMIDT. While the first *facu* culture 4675 between a *falcataria* female

and a *curvatula* male from the parish of Snappertuna gave 65 ♂♂ and 59 ♀♀, and all the fixed gonads of the caterpillars were pure testes or ovaries, of five fixed larval gonads of the culture 4770 four exhibited a clear transformation of ovaries into testes, and only one caterpillar had normal testes, which possibly had completed their reversal. The mother of the culture 4770 was a *falcataria* female from the vicinity of the town of Borgå, while the *curvatula* male belonged to the Snappertuna population. The two localities where the *falcataria* moth was found lie 110 km. apart. A more pertinent fact seems, however, to be that the Snappertuna caterpillars were collected on the outermost islands, whereas the *falcataria* female came from the inland of the Borgå region. Whether we actually here have races of different strength in the sense of GOLDSCHMIDT, cannot be decided before further crossing experiments have been made. It is unfortunately not very easy to obtain material of these relatively rare moths.

The transformation of the very young ovaries into testes is rather similar to the cases described in *Lymantria* by GOLDSCHMIDT. It seems obvious that the ovaries here described would also have been completely transformed into testes. This is supported by the occurrence of the normal testis and the testis which still showed traces of ovarial character. It is possible that the reversible females would have been transformed into fertile males, and control backcrosses would have been possible between these and the pure parental species. I hope to be able to carry out such experiments in the summer of 1948. In any case it is obvious that the turning point — to use GOLDSCHMIDT's terminology — lies very early in the development, since even very young ovaries with undifferentiated young oocytes in pachytene are distinctly beginning to be transformed. Unfortunately it does not seem possible to analyse these cases more in detail, this being prevented by the irregular chromosome combinations, and especially by the fact that it is impossible to decide how the sex chromosomes of the parental species behave at the pairing stage.

It is in accordance with expectation that the hybrid males show considerable *sterility*, as pairing conditions of the type found in the hybrids *cufa* and *facu* (*cf.* Tables 2 and 3) have proved especially unfavourable in regard to fertility in other Lepidoptera hybrids. Either ought all the chromosomes to pair, or pairing ought to fail almost completely to give sperms which are able to function. The chromosomes of the sperms must in addition give a harmonious and viable combination with the egg chromosomes. In other words, haploid/hyperhaploid or

diploid/subdiploid chromosome numbers would be most favourable. A partial pairing with a wide range of variation is the most unfavourable type, and it is just this which is characteristic of the *Drepana* hybrids. Tables 2 and 3 are very convincing in this respect. The chromosome numbers vary from 31 to 55, the different individuals having quite dissimilar numbers. Spermatocytes with the numbers 37, 40 and 45 are — peculiarly enough in both hybrids — most frequent.

The only backcross of a *cufa* ♂ with a *falcataria* ♀ gave only 2,7 % caterpillars, and of the eight F_2 cultures only two yielded caterpillars, 0,7 % and 1,0 % respectively. (*Cf.* Table 1.)

Helsingfors, May, 1948.

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NEW CASES OF SEGMENTAL INTERCHANGE AND SOME OTHER MEIOTIC IRREGULARITIES IN RYE

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DURING a stay in Ecuador one of us (MÜNTZING) got hold of a variety of rye (*Secale cereale*) cultivated in the mountainous parts of the country under the name of »Trigo nacional»[1]. The kernels of this variety, which has probably been cultivated in Ecuador for a long time, were very small and similar to the kernels of inbred strains of rye. It is not known whether this variety is of winter or summer type, *i. e.* whether it needs a period of low temperature or not in order to shoot. In 1947, however, it was sown in Svalöf in spring, developed fairly well and flowered in June. Next year it was sown in the same way as a summer rye, but this year it was very slow in development and many plants remained at the vegetative stage. Hence it may need a period of low temperature during its early development in order to shoot. In the autumn of 1948 it was tentatively sown as a winter rye.

In 1947 some crosses were undertaken between the Ecuadorian type as female parent and a Swedish variety of summer rye as male parent. Of ten ears crosspollinated, only four gave a variable degree of seed-setting. The seeds of the hybrids were sown in the spring of 1948 together with seeds of both parent types, and the resulting plants were grown together in a frame. The morphological differences between the parents were large enough to show that the supposed F_1 plants were in fact true hybrids. From this material fixations of meiosis were undertaken, the fixative used being chrome-acetic-formalin with prefixation in Carnoy. A total of 9 fixations were made of the F_1 plants, the corresponding numbers of the mother and father strains being 12 of each. The fixed material was embedded in paraffin and the slides stained with crystal violet.

1. *Cases of segmental interchange.* — Two hybrid plants were found to have an association of 4 chromosomes at first metaphase. At

[1] For procuring this seed material we are much indebted to Mr. BJARNE STAËL VON HOLSTEIN.

first this was taken as evidence of a structural differentiation between the parent strains. This was not the case, however, associations of 4 chromosomes also being present in the mother strain. More precisely, the occurrence of this phenomenon was as follows:

For the crosses 4 different individuals of the Ecuadorian rye were used as female parents, *viz.* the plants 495/1, 495/3, 495/13 and 495/17.

Plant 495/1 gave rise to a number of hybrid plants, 4 of which were cytologically examined and found to have 7 bivalents at first metaphase.

Plant 495/3 gave one hybrid plant, which on cytological examination was also found to be normal, having 7 bivalents.

Plant 495/13 was the mother plant of 3 F_1 individuals. Two of these had 7 bivalents, the third one had an association of 4 chromosomes + 5 bivalents at meiosis.

Plant 495/17 gave rise to a single hybrid plant also having $1_{IV} + + 5_{II}$ at meiosis.

The occurrence of F_1 plants with and without an association of 4 chromosomes might be due to heterozygosity for a segmental interchange either in the female or in the male parent. In the present case the females were found to be responsible. This was evident from a cytological examination of progenies after open pollination of the male as well as female parents. In the offspring of plant 495/13 two individuals were examined, one of them having $1_{IV} + 5_{II}$, the other one 7_{II}. In the offspring of plant 495/17 4 individuals were examined, all having $1_{IV} + 5_{II}$.

The mother plants giving exclusively normal hybrids with 7_{II} also gave daughter plants which, after open pollination, showed the same regularity. More precisely, plants 495/1 and 495/3 gave 3 daughter plants each, all having 7_{II} at meiosis.

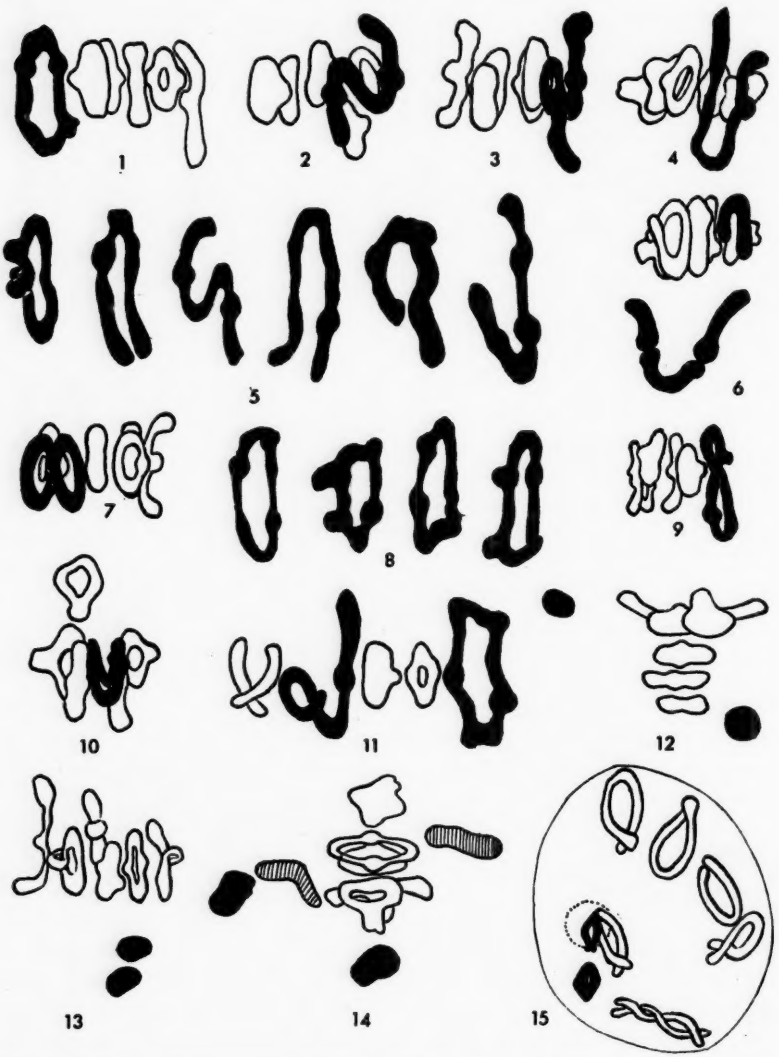
For the crosses pollen was collected from several plants of the same plot, four different plots of the same variety being used. Seeds from these 4 plots were germinated, and a total of 12 daughter plants were cytologically examined. All of these plants were found to have a regular meiosis. Hence, the occurrence of heterozygosity for a segmental interchange could be traced to two different and unrelated plants of the Ecuadorian variety. This heterozygosity caused the occurrence of plants with $1_{IV} + 5_{II}$ in the offspring after open pollination as well as in the hybrid progenies. The plants with $1_{IV} + 5_{II}$ derived from 495/13 may be said to belong to Group I, plants of the same kind derived from 495/17 belong to Group II.

The appearance of the »quadrivalents» of *Group I* may be seen from Figs. 1—6. Figs. 1—2 represent first metaphase configurations from the mother strain. In Fig. 1 the quadrivalent is a non-zigzag ring, in Fig. 2 it is a zigzag chain. In this plant 217 I-M groups were observed, all of them containing $1_{IV} + 5_{II}$. In 169 cells the quadrivalent was ring-shaped, in 48 cells it was a chain. Figs. 3—6 are from a hybrid daughter plant, in which chain quadrivalents were prevalent. Of 170 I-M groups studied, 27 contained rings, 130 chains, and in 13 cells the four chromosomes involved in the translocation were represented as $1_{III} + 1_{I}$ (Fig. 6). Thus, in the hybrid daughter plant the chiasma frequency in the quadrivalent was somewhat lower than in the mother plant. Otherwise the appearance of the quadrivalents was just the same. In Figs. 3—4 there is a zigzag and a non-zigzag chain, respectively, in addition to the five bivalents. Fig. 5 represents 6 chain quadrivalents of Group I separately drawn.

Group II is represented by Figs. 7—11. Fig. 7 shows a I-M group in which the association of four chromosomes is represented by a zigzag ring. Fig. 8 shows the appearance of 4 non-zigzag rings separately drawn. Figs. 7—8 are from various daughter plants obtained from plant 495/17 after open pollination. Figs. 9—11 represent a hybrid between 495/17 (Ecuadorian) and the Swedish rye. Figs. 9 and 10 show the normal configurations in this hybrid, $1_{IV} + 5_{II}$, but Fig. 11 is quite exceptional, this metaphase containing the configuration $2_{IV} + 2_{II} + 2_{I}$.

It is not yet clear whether the cases of segmental interchange occurring in Groups I and II are identical or not. The original mother plants were raised from the same seed sample of the Ecuadorian rye. It is possible that this seed sample was derived from a single field but this is not known. At any rate, the sample contained at least 20,000 seeds and, hence, it is rather unlikely that the two plants in question were closely related.

A comparison between the quadrivalent configurations in Groups I and II (Figs. 1—6 and 7—11 respectively) does not reveal any clear difference. However, if it is a question of the same segmental interchange, this interchange should be rather frequent in the Ecuadorian population, as it was met with in two unrelated plants. This possibility was tested by a study of meiosis in 17 plants, derived from the original seed sample. Meiosis in all these plants was normal, no associations of 4 chromosomes being present at first metaphase. Thus, it seems likely that the interchanges occurring in Groups I and II involve differ-



Figs. 1—6. Evidence of segmental interchange in Group I. Figs. 1—2, I-M groups with $1_{IV} + 5_{II}$ in a plant of the Ecuadorian variety; Figs. 3—4, I-M groups from F_1 plants, also with $1_{IV} + 5_{II}$. Fig. 5, 6 »quadrivalents» of Group I, separately drawn. Fig. 6, a deviating configuration in an F_1 plant, $1_{III} + 1_I + 5_{II}$. Figs. 7—11. Evidence of segmental interchange in Group II. Fig. 7, $1_{IV} + 5_{II}$ at I-M in the mother strain. Fig. 8, 4 »quadrivalents» from the mother strain, separately drawn. Figs. 9—10, I-M

ent chromosomes. This will of course be tested by crosses between interchange plants belonging to the two groups.

Cases of segmental interchange in rye have previously been described by DARLINGTON (1933) and by MÜNTZING and PRAKKEN (1941). In the latter paper four different cases are described and of these the so-called type *a* shows most similarity to the present cases. The similarity concerns the morphological appearance of the quadrivalent associations, their regular occurrence and the preponderance of zigzag arrangements at first metaphase. In the present material the various kinds of quadrivalents were found to occur in the following frequencies (Table 1).

TABLE 1. *Types of quadrivalents.*

Category	Ring		Chain		n	Per cent rings	Per cent zigzag arrangements
	zz	non-zz	zz	non-zz			
Group I, mother strain	108	61	34	14	217	77,9	65,4
» , F_1	26	1	122	21	170	15,9	87,1
» II, mother strain	40	77	39	27	183	63,9	43,2
» , » »	48	52	75	31	206	48,5	59,7
» , » »	67	13	7	5	92	87,0	80,4
» , » »	135	63	16	10	224	88,4	67,4
» , F_1	86	48	38	18	190	70,5	65,3

In 5 of the 7 plants examined rings are more frequent than chains, and in 6 of the 7 plants zigzag arrangement is more frequent than non-zigzag arrangement. There is a rather marked inter-plant variation, however, the causes of which are unknown. The variation in chiasma frequency, normally occurring in rye populations (*cf.* MÜNTZING and AKDIK, 1948) may account for part of the differences, as, *e. g.*, between the two plants of Group I (Table 1).

In the offspring of plants heterozygous for a segmental interchange 50 per cent of the individuals should be heterozygous for the interchange and show an association of four chromosomes at meiosis. The 5 plants belonging to Group I were distributed as follows:

groups with $1_{IV} + 5_{II}$ in an F_1 plant. Fig. 11, an exceptional I-M group from the same F_1 plant having $2_{IV} + 2_{II} + 2_I$. — Figs. 12—14, occurrence of ring-shaped univalents in a local sector. Figs. 12—13, $6_{II} + 2$ ring univalents. Fig. 14, $5_{II} + 2$ rod univalents (cross-hatched) + 2 ring univalents (black). Fig. 15, diakinesis in the same plant. Probably 6 normal bivalents + 2 smaller bodies (black) representing the ring univalents. — × 2000.

	7_{II}	$1_{IV} + 5_{II}$
Mother strain	1	1
F_1	2	1

The numbers are small but are in accordance with the 1 : 1 expectation. In Group II the following values were obtained.

	7_{II}	$1_{IV} + 5_{II}$
Mother strain	0	4
F_1	0	1

Thus, all 5 plants examined were heterozygous for the interchange. The probability that this corresponds to a 1 : 1 ratio is rather low, P being 0,03. Further work is required to decide whether there is a significant preponderance of interchange heterozygotes in this material. At any rate, the present material is different in this respect from the type *a* heterozygotes previously studied by MÜNTZING and PRAKKEN (1941). In this case there was a significant and very marked deficiency of plants heterozygous for the interchange, the great majority of the daughter plants having 7_{II}.

2. *Ring-shaped univalents.* — In a plant belonging to the Ecuadorian variety most of the anthers showed a normal meiosis with 7 bivalents at I-M. In one loculus, however, almost all the p.m.c.'s contained 6 bivalents and 2 univalents. Of 125 cells in this loculus 95 per cent showed $6_{II} + 2_I$ (or occasionally a higher number of univalents), the remaining 5 per cent containing 7_{II}. The univalents in this case were not of the usual rod type but evidently closed rings, at this stage appearing as discs without clearly visible holes (Figs. 12—13). The appearance of the discs was of course different when they were seen from the flat side or more or less from the edge. They were similar to the ring-shaped large iso-fragments previously observed by MÜNTZING (1944, Figs. 20—29) but differed from this case by always being closed. Fig. 14 is especially instructive, as this cell also contains 2 rod univalents of the usual type, the total configuration being $5_{II} + 2\ rod_I + 2\ ring_I$. Unfortunately no anaphase stages were available and, thus, it is not known with certainty whether the ring univalents are true rings or iso-chromosomes paired with themselves. The latter alternative is most plausible, as 5 per cent of the cells contained 7 normal bivalents, thus showing that the univalents may occasionally form bivalents with each other.

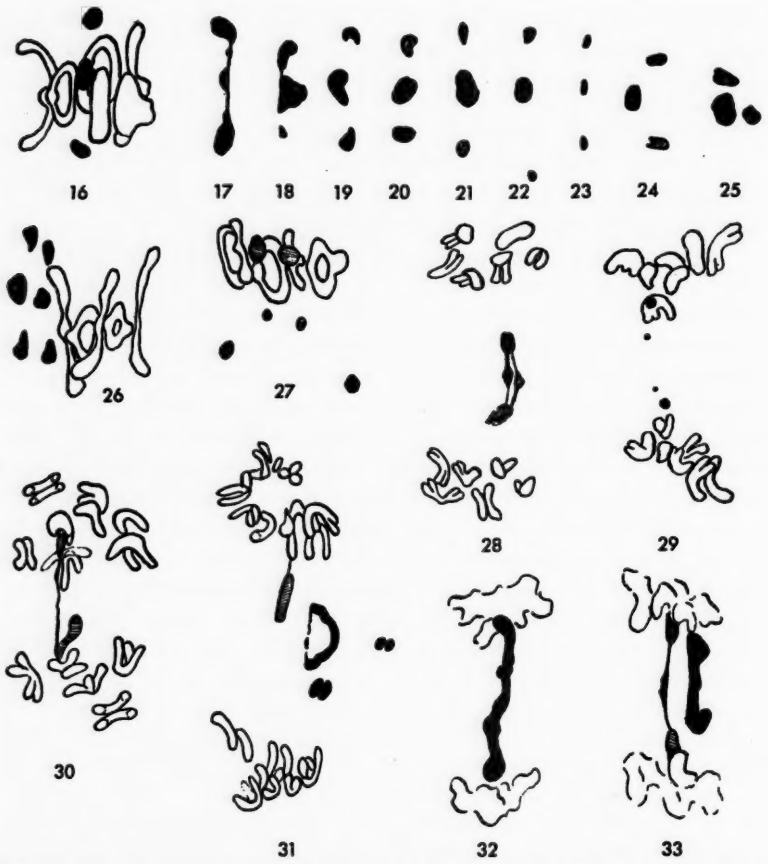
In another slide of the same plant diakinesis stages were seen

which apparently contained 6 normal bivalents + 2 smaller and ring-shaped chromosomes (Fig. 15). These chromosomes may correspond to the ring chromosomes observed at first metaphase. This is somewhat uncertain, however, as other evidence was obtained that one of the normal bivalents at diakinesis may sometimes be composed of two separate parts apparently free from each other but in reality connected by invisible fibres. At any rate, the local occurrence of p.m.c.'s containing 6 normal chromosomes and 2 ring-shaped ones is quite certain.

The origin of these peculiar chromosomes is uncertain. Evidently they have nothing to do with B-chromosomes but are derived from one of the ordinary A-chromosomes. The 2 ring-shaped univalents in the same cell were always of equal size and are probably identical. This identity is supported by their ability of occasional pairing with each other. The most plausible origin seems to be by misdivision of one member of a pair of A-chromosomes, followed by loss of one arm and doubling of the iso-chromosome derived from the other arm. Unfortunately there is no hope of recovering this interesting chromosome type in the offspring, as it was restricted to a rather small local sector.

3. *Apparent fragmentation of bivalents.* — In one plant of the Ecuadorian variety one or two rod bivalents behaved in a peculiar way at I-M and I-A as illustrated by Figs. 16—29. In Fig. 16 there are 6 normal metaphase bivalents and 3 apparently separate bodies, which correspond to the 7th bivalent. The three bodies evidently represent the two terminal parts of the chromosomes and the chiasma region, the possible connections between these parts being completely invisible. When different metaphase groups were compared all transitions could be seen between almost normal bivalents (Fig. 17) and cases in which the three components of the bivalent were apparently quite free from each other (Figs. 24—25). An intermediate group is represented by those bivalents in which the relative position of the three bodies is normal but no connections between these parts are visible (Figs. 16, and 19—23). Correlated to these changes is an apparent chromatin »diminution», the three bodies representing the bivalent sometimes being quite small (Fig. 23).

This phenomenon was only observed in one plant, but in this plant it was frequent. Of 100 metaphase groups observed, 37 contained one bivalent of this kind and 7 contained two such bivalents (Figs. 26—27). At first anaphase there seems to be a high frequency of fragmentation but in most cases this fragmentation is probably only apparent. In Fig. 28 a delayed anaphase separation of such a bivalent may be seen.



Figs. 16—29. Apparent fragmentation and chromatin diminution of bivalents. Fig. 16, I-M with 6 normal and 1 abnormal bivalent, composed of 3 apparently quite separate parts. Figs. 17—25, »fragment bivalents», separately drawn. In Fig. 17 it is almost normal, in Figs. 24—25 the three bodies representing the bivalent appear to be quite free from each other. In Fig. 23, an extreme case of chromatin »diminution» also more or less visible, *e. g.*, in Figs. 21, 22, 27. Figs. 26—27, 5 normal + 2 fragment bivalents. In Fig. 27 the bodies representing the two abnormal bivalents are far from each other and at different levels. Fig. 28, lagging fragment bivalent showing probable mode of later separation. Fig. 29, a later stage probably corresponding to Fig. 28. Apparent fragmentation. — Figs. 30—33, various irregularities at I-A in some F_1 plants. — × 2000.

It is rather obvious that one larger and one smaller body united by a thin strand will go to each pole, and this will probably give rise to such

structures as the apparent fragments in Fig. 29. We are inclined to believe that also at metaphase there are always or mostly thin strands connecting the parts which appear to be separated. The reason for this anomalous behaviour is unknown, but a deficit of nucleic acid especially affecting one or two members of the genome may be suggested. However, other cases of a probable deficit of nucleic acid in rye have been met with (MÜNTZING and AKDIK, 1948, pp. 495—500) in which the deficit affected all the chromosomes and not special bivalents.

4. *Other anomalies.* — Irregularities at first anaphase were observed in some plants as shown by Figs. 30—33. In one F_1 plant, having an association of four chromosomes at first metaphase, bridges, fragments and apparent ring chromosomes were observed (Figs. 30—31). In another F_1 plant one or two bridges without fragments were rather frequent (Figs. 32—33). The causes of these deviations are not known and further studies of new hybrid material are necessary in order to decide whether structural differences between the parental varieties are responsible for these anomalies.

SUMMARY.

(1) A primitive strain of rye from Ecuador was crossed with Swedish summer rye and meiosis observed in the F_1 and parent strains. In some F_1 plants and also in plants of the Ecuadorian strain cases of segmental interchange were studied.

(2) In the offspring of plants heterozygous for an interchange the frequency of daughter plants with associations of four chromosomes was higher than expected and significantly higher than in a previous case of rye interchange studied by MÜNTZING and PRAKKEN.

(3) In one plant of the Ecuadorian variety a local sector contained pollen mother cells with 6 bivalents + 2 mostly unpaired and ring-shaped univalents. The most plausible origin of these rings is mis-division of a chromosome at mitosis followed by loss of one arm and doubling of the iso-chromosome derived from the other arm.

(4) At metaphase of meiosis in another plant of the Ecuadorian variety one or two rod bivalents frequently appeared to be fragmented into three parts, corresponding to the two terminal parts and the chiasma region. Correlated to these changes is an apparent chromatin »diminution», the three bodies representing the bivalent sometimes being quite small. In most cases the apparently separate parts of the bivalents are probably connected by thin strands, which do not take the stain.

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THE STRUCTURE OF THE CENTROMERE OF THE CHROMOSOMES OF RYE

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DURING a study of the B chromosomes of rye at pachytene (LIMA-DE-FARIA, 1948) and a study of the A complement, also at pachytene (LIMA-DE-FARIA, unpublished), the writer has been able to observe the existence of a definite structure in the centromere of these chromosomes.

In my paper of 1948 I described the centromere region of the standard fragment (a type of B chromosome) as »a very distinct gap without clear chromomeres». This description of the centromere region was based on observations made in slides of average staining. Both the centromere regions of the A and of the B chromosomes, in these slides, are apparently a structureless gap that is only faintly stained. As the observed intensity of stain in the centromere region was similar to that sometimes observed between the chromomeres (for instance, the parts on both sides of the knob of the standard fragment), I had already suspected a possible existence of chromomeres at the centromere, and for this reason I described the centromere region as cited above.

Material and methods. — The plant studied originated from the cross between an inbred line and »Vasa» rye (a commercial variety), the latter parent plant having two standard fragments. This plant belongs to the material cultivated by Prof. MÜNTZING at Svalöf. The writer wishes to express his thanks to Prof. MÜNTZING for the material kindly ceded him and to Amanuensis N. NYBOM for help in making the microphotographs.

The technique used has been described earlier by LIMA-DE-FARIA (1948). It will only be mentioned here that the material was fixed in acetic-alcohol 1 : 4 for four hours, transferred to 95 % alcohol (overnight), stored in 70 % alcohol and stained with aceto-carmine (with iron). No pre-treatment was used.

In two slides that were more deeply stained than the common slides, and in which the structure of the chromosomes was still more distinct than in the average ones, the centromere region was observed to contain chromomeres as well.

Observations. — The centromere appears as a region very distinctly separated from the arms of the chromosome.

In all the chromosomes the region of the arms close to the centromere has been found to show thicker and more deeply stained chromomeres than the rest of the arms. This thicker and deeply stained region is not easy to delimit on the opposite side of the centromere because, on this side, there is a continuous transition. In Fig. 1 it can be seen that this region occupies about one-fourth of each arm. Especially the pair of chromomeres close to the centromere are distinctly thicker in this chromosome.

Fig. 1, camera lucida drawing of a pair of A chromosomes at pachytene, with one knob at each end. To the right, the centromere region with the two pairs of centromeric chromomeres. — × 2500.

The centromere region (Figs. 1, 2 and 3) has a compound structure. In the pairs of chromosomes observed at pachytene it is possible to recognize three distinct zones in the centromere region, *viz.*

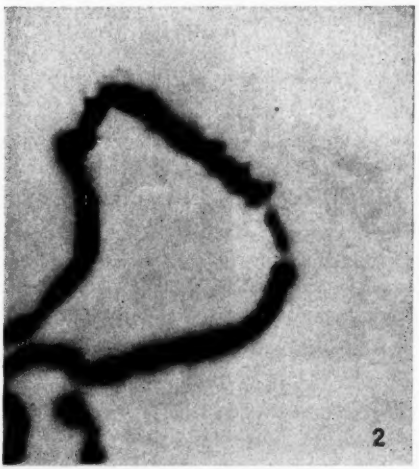
(1) A first zone, the exterior zone, in which the fibrillae are almost unstained.

(2) A second zone, the chromomeric zone, composed of two pairs of centromeric chromomeres. As Fig. 1 represents a pair of chromosomes, it shows that the centromere of each chromosome possesses two chromomeres disposed in longitudinal sequence.

(3) A third zone, the interior zone, comprises the space between the two pairs of centromeric chromomeres. This zone is characterized by a deeper stain than the first zone.

The exterior zone is always unstained or very faintly stained; no structure can be distinguished except two thin fibrillae (corresponding to the two chromosomes) connecting the last pair of chromomeres of the arms with the centromeric chromomeres.

The centromeric chromomeres are quite distinct (Figs. 1—7). Their distinctness with reference to the two chromosomes paired could be very well seen, and their distinctness with reference to the longitudinal differentiation inside the centromere could also be seen quite clearly (especially Figs. 2 and 7). Whether these two centromeric chromo-



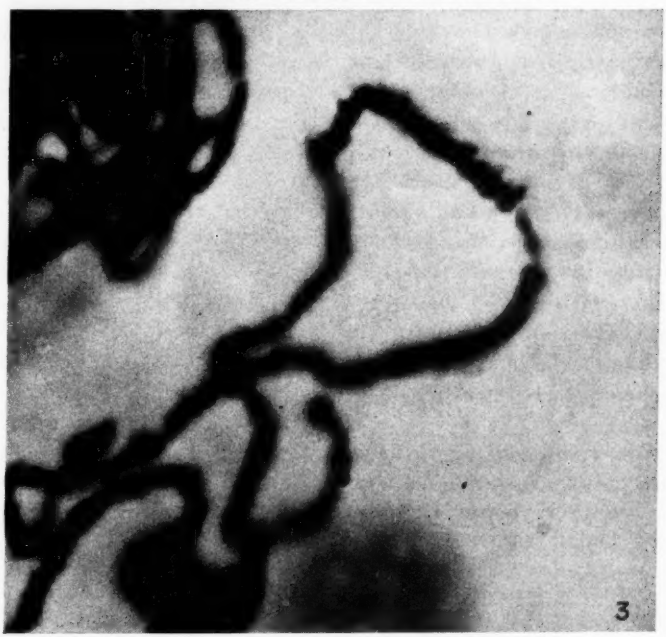


Fig. 2, photograph of a part of the pair of A chromosomes drawn in Fig. 1, showing especially the centromere region with the two pairs of centromeric chromomeres very distinct. — × 3600. — Fig. 3, photograph of the pair of A chromosomes shown in Fig. 1. — × 3600.

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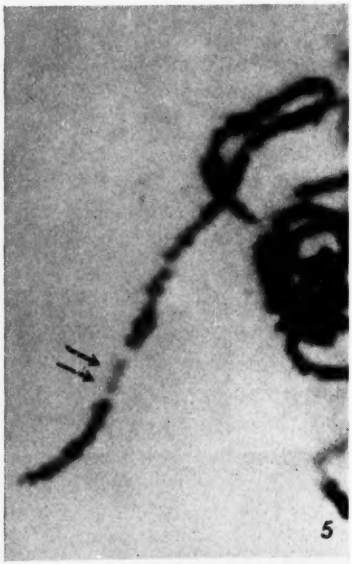

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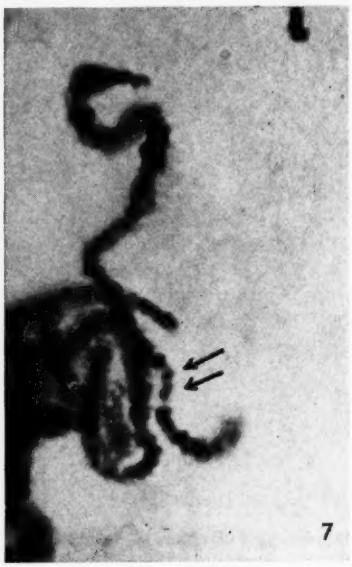

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meres (considering now a single chromosome) still have compound structures, could not be observed.

The interior zone connecting the centromeric chromomeres is always definitely more stained than the exterior zone. Only two thin fibrillae (corresponding to the two chromosomes) could be observed. I am inclined to think that this zone may be resolved into centromeric chromomeres of minor size, though with the present technique I have been unable to distinguish anything of this nature (see below).

Considerations. — The names given to the different zones must not be regarded as new terms but merely as expressions suitable for the present description. Probably more suitable terms can be found when further details are known about the structure of the centromere region in rye.

I have avoided the use of the term »spindle spherule» merely because the observations were made at prophase, and have employed the expression centromeric chromomeres instead for two reasons. First, because the formations observed in the centromere region are apparently quite similar to the fainter chromomeres observed in the arms of the chromosomes, and second, because according to NEBEL's (1939) definition of chromomeres, this term is simply morphologic. Hence it does not imply that the centromeric chromomeres are of the same nature as the chromomeres of the arms. Most probably they are not.

If we try to compare these observations with those of other authors, we realize that the observations of ÖSTERGREN are those which resemble most the present ones. ÖSTERGREN (1947) has observed in pre-treated root tips, at metaphase and at early prophase, two spindle spherules corresponding to the two chromatids. He has been able to ascertain that the spindle spherules were double. These two spindle spherules in each chromatid can correspond to the two centromeric chromomeres of each chromosome of rye.

On account of the coiling of chromonemata that takes place between pachytene and metaphase it is reasonable to assume that at this later stage the two centromeric chromomeres stay very close to one

Fig. 4, camera lucida drawing of a pair of standard fragments at pachytene. Note the subterminal knob at the end of the long arm and the presence of the two pairs of centromeric chromomeres. — × 2000. — Fig. 5, photograph of the pair of standard fragments drawn in Fig. 4. The two pairs of centromeric chromomeres indicated by two arrows. — × 2900. — Fig. 6, camera lucida drawing of another pair of standard fragments. — × 2500. — Fig. 7, photograph of the pair of standard fragments drawn in Fig. 6. The two pairs of centromeric chromomeres, here very distinct, are indicated by two arrows. — × 3000.

another, as in the case of ÖSTERGREN's observations, or are impossible to distinguish from each other as in the case of SCHRADER's and KOSLOV's observations.

In this way, the spindle spherule of each chromatid observed by SCHRADER (1939) in Amphibia can be interpreted by assuming close contact of two centromeric chromomeres. The exterior region observed here in rye would correspond to his commissural region or cup, but this homology is more difficult to establish with certainty.

The same assumption can be made in regard to the work of KOSLOV (1937). He also observed in the mitotic chromosomes of *Fritillaria* a spindle spherule corresponding to each chromatid, inserted in a commissural region.

The homology between the observations of LEVAN (1946) in *Allium* and these in rye is more uncertain, but his case of *Dipcadi*, after cold treatment, in which he observed two strongly stained granules corresponding to the two chromatids, can be interpreted in the same way.

SCHRADER (*l. c.*) and ÖSTERGREN (*l. c.*) have pointed out that, owing to the fact that the actively mobile component of the centromere is divided at metaphase and already at prophase, the property of the centromere of remaining undivided until the end of metaphase of mitosis and until the second division of meiosis must be carried by a material part that is not the mobile component.

This property of holding together the chromatids can only be ascribed either to the commissural region or to some component existing between the spindle spherules. The fact that KOSLOV (*l. c.*) has also seen the commissural region double at metaphase strongly argues against the attribution of this property to the commissural region. Therefore, this property can be ascribed to the zone now found in rye between the centromeric chromomeres.

We may thus ascribe to the three zones of the centromere region of the chromosomes of rye the following properties, *viz.*

(1) Exterior zone, connection with the arms.

(2) Chromomeric zone, active mobility.

(3) Interior zone, special cycle of division.

This structure of the centromere of the rye chromosomes helps us to understand two phenomena for which no or little structural evidence has been previously available.

The first is the mitotic behaviour of chromosome fragments obtained by breaks through the centromere in maize after X-raying

(McCLINTOCK, 1938; RHOADES and McCLINTOCK, 1935). If we assume that the X-ray breakage has taken place at prophase through the interior zone of the centromere, the resulting centromere fragments would possess (1) one centromeric chromomere which would allow of the mobility of the new chromosome fragment and (2) a part of the interior zone which would maintain the mechanism of division at the proper time. Then the mitotic movements observed in these chromosome fragments in successive generations of cells would not be surprising.

The second phenomenon that it helps us to understand is misdivision.

ÖSTERGREN (*l. c.*) has developed the idea that the possible existence of a chief transversal doubleness of the motoric apparatus of the centromere in relation to the longitudinal doubleness would lead to an abnormal orientation of univalents in the spindle perpendicular to the normal mitotic one as well as to a transverse division of the centromere.

The present finding in rye gives a strong support to ÖSTERGREN's interpretation. An A chromosome that gets an abnormal orientation in the spindle would break at the interior zone along a plane corresponding to its orientation. In any case the resulting centromere fragments would carry a centromeric chromomere, which would maintain the mobility of the new chromosome fragment and a part of the interior zone, which would permit them to divide normally in subsequent cell generations (it is for this reason, combined with the fact that the interior zone is more deeply stained, that I am inclined to consider that this zone also has a compound structure). This explanation of misdivision supported by the structure of the centromere of rye chromosomes is corroborated by the fact that misdivision and the production of iso-chromosomes was observed previously in this organism.

The cytological observation of misdividing A chromosomes of rye was reported by LEVAN (1942) in haploid rye and by PRAKKEN (1943), who observed »transverse division of lagging chromosomes» (p. 486). MÜNTZING (1944) observed misdivision in the B chromosomes (large iso-fragment) as well.

The existence of iso-chromosomes in plants of rye was shown by MÜNTZING (1944) on the basis of the size and pairing behaviour of »chromosome fragments» (large iso-fragment and small iso-fragment) derived from another type of B chromosome, the »standard fragment» (Figs. 4—7). The confirmation that the large iso-fragment, studied by MÜNTZING, is derived from the doubling of the long arm of the standard fragment has been obtained by an analysis of the structure of these two

types of chromosomes at pachytene (MÜNTZING and LIMA-DE-FARIA, in the press).

To sum up, the present idea of the mechanism of misdivision in rye is supported by three sources of observations: longitudinal differentiation of the centromere region in three zones in A and B chromosomes, observation of transverse division of lagging A chromosomes, and analysis of the structure of iso-chromosomes at pachytene.

The explanations suggested for the mechanism of misdivision by DARLINGTON (1939), by NEBEL (1939) and by MÜNTZING (1946) had no support in any observed structure of the centromere. As the present finding in rye does not give them any support, the mechanisms proposed by these authors ought to be considered uncertain.

GILES (1944) has explained the existence of metacentric chromosomes in *Gasteria* on the basis of crossing-over in a heterozygous inversion involving the centromere. This explanation suits his results but does not find support in the case of rye. First, because of the evidence stated above of a real misdivision of the centromere, second, because misdivision has been observed in rye in univalents (PRAKKEN, 1943), and third, because MÜNTZING (1944) has observed that »the frequency of new fragment chromosomes seems to be much higher in plants with a single extra fragment than in plants with two or more extra fragment chromosomes».

SUMMARY.

The study at pachytene of A and B chromosomes of *Secale cereale* L. reveals that the centromere region has a compound structure with distinct chromomeres.

Three zones can be distinguished in this region in each chromosome: an exterior zone, a chromomeric zone, composed of two centromeric chromomeres, and an interior zone.

The properties of these zones and their homology with previous observations of other authors are discussed. The conclusion is drawn that we may ascribe to the three zones the following properties, respectively, *viz.* (1) Connection with the arms. (2) Active mobility. (3) Special cycle of division.

The behaviour of fragment chromosomes of maize arising after breakage through the centromere and the mechanism of misdivision in rye are interpreted on the basis of these observations.

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INVESTIGATIONS ON SECONDARY CONSTRICTIONS IN POLYGONATUM

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I. INTRODUCTION.

DURING a previous study on the chromosomal conditions in *Polygonatum* species belonging to Group *Alternifolia* BAKER (SUOMALAINEN, 1947), attention was drawn to the exceptionally long secondary constrictions in certain chromosomes of these plants. It has been pointed out by DARLINGTON (1937, p. 39) that long secondary constrictions separating two large chromosome segments are very rare. It seemed therefore of interest to subject these long constrictions to a closer examination, especially as they also deviated in other respects from the commonly presented type. As already described earlier (SUOMALAINEN, *l. c.*), the constrictions in *Polygonatum* as a rule appear as unstained gaps in the chromosomes without a distinct connecting thread, which is usually seen in drawings representing SAT-chromosomes. It was therefore tempting to try to elucidate the actual structure of these secondary constrictions on the basis of the material available. Since the chromosome morphology of the various *Polygonatum* species has been dealt with rather extensively in the previous paper, to which reference may be made in regard to all details, it will not be reconsidered in this connection, except in so far as it has a bearing upon the question of secondary constrictions. In the following the terms SAT-constriction and SAT-chromosome are used in the sense of HEITZ (1931 a and b).

II. MATERIAL AND METHODS.

The plant material already dealt with in my earlier paper (SUOMALAINEN, 1947) has also been used for the present study, completed with material from *Smilacina stellata* (Botanical Garden of Helsinki University) for the sake of comparison. Partly even the same slides have been re-examined. These slides have, however, been amplified by a great number of new ones, with the use of different fixing and staining methods. To eliminate, so far as possible, the effect of artefacts material prepared with different techniques has been studied. This seemed to be

of special importance, as secondary constrictions are known to be rather labile in regard to their fixation and staining properties.

In the following table the different fixation and staining combinations are given together with the abbreviations used in the legends of the figures.

Fixatives	Stains: Aceto-lacmoid	Aceto-orcein	Feulgen (F)	Crystal violet (CV)	Unna-Pappenheim
Aceto-alcohol (AA) ..	+	+	+		+
Craf			+	+	
Lewitsky			+	+	
Zenker					+
2BE			+	+	
2BD			+	+	
Benda (B)			+	+	

Paraffin sections (25 μ) as well as squash preparations have been made of the root tips. The fixatives and staining fluids have been prepared and used according to the formulae as given by DARLINGTON and LA COUR (1942) and LA COUR (1947). It may be mentioned already in this connection that the results obtained with the use of different preparation methods agree in principle. The slides prepared with the aid of the different techniques are, however, most variable in regard to their quality. The best preparations have been obtained by using LA COUR's 2BD for fixation and Feulgen or crystal violet for staining. Almost as satisfactory results have been achieved with 2BE and Benda. Of the fixatives which do not contain osmic acid, Lewitsky's fluid without acetic acid revealed best the secondary constrictions. When Craf-solution was used the chromosomes appeared to be somewhat more swollen. Good results, especially in regard to secondary constrictions, have also been gained with the squash method. In this case the root tips were fixed in aceto-alcohol (1 : 3) for 12 hours and stained in bulk with aceto-lacmoid, aceto-orcein, or Feulgen, after which they were squashed. Contrary to the effect of other fixatives, material treated with Zenker's formol exhibited a rather bad fixation, the chromosomes appearing swollen and clumped. Nor was the subsequent methyl-green-pyronine staining very successful, failing as it did to show any additional features in the chromosomes as compared with slides prepared according to the other methods. Special care was taken in regard to the Feulgen staining, as being specific for thymonucleic acid, this method

being used as described by LA COUR (1947). For the discrimination of nucleoli longitudinal sections have been made of root tips and stained either with crystal violet or Feulgen, the latter combined with light green.

To reveal the existence of possible heterochromatic chromosome segments in the sense of DARLINGTON and LA COUR (1938, 1940), a number of *Polygonatum* species have been subjected to cold-treatment. The plants have been kept at a temperature of 0—4° C for 3 and 6 days respectively, after which root tips have been fixed with the use of different fixing fluids. In October, 1947, squash preparations were made after frosty nights of several other *Polygonatum* species for the same purpose. In none of these preparations, however, could any heterochromatic segments be detected.

The method of LEVAN (1946) for the demonstration of heterochromatin in plant chromosomes has also been tried. According to this, root tips have been prefixed for a few hours in 0,005 mol, mercuric nitrate solution, and thereafter fixed in Craf and stained in crystal violet. In the various *Polygonatum* species and in *Smilacina stellata* this method did not exhibit any differentially stained chromosome segments; nor were the fixation and staining in general in these plants satisfactory.

The magnification of the figures reproduced in this paper is 3750 ×.

III. OBSERVATIONS.

As has been described in my paper on *Polygonatum* species belonging to the group *Alternifolia* (SUOMALAINEN, 1947) the diploid species as a rule possess two pairs of chromosomes provided with secondary constrictions. These constrictions appear as rather long and unstained, clear-cut gaps in the chromosomes. The statement advanced earlier (SUOMALAINEN, *l. c.*) that no trace of any connecting thread has been observed must, however, be modified, for it has become apparent that, though the »SAT-thread» is not present as a thin filament, there is often to be seen a broad, faintly stained zone sometimes equal in breadth to the chromosome diameter. This faintly stained zone is represented in the adjoining figures, the respective parts being stippled. It must, however, be pointed out that in most cases this zone is completely unstained, the examples presented here being in many cases chosen especially because they reveal a staining. The degree of contraction of the SAT-zone is very variable. It could be established that

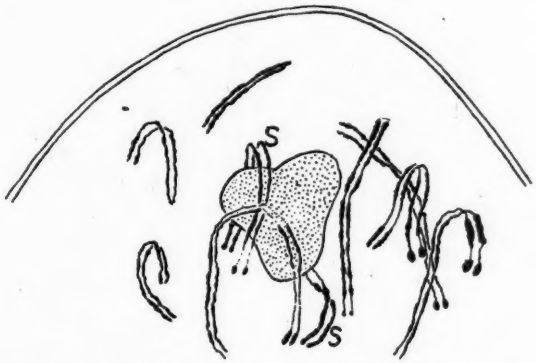

Fig. 1. *P. officinale* (Piikkiö). Prophase of a PG mitosis (n = 10). The two chromosomes marked with S are attached to the nucleolus (2BE, CV).

there exists a correlation between the contraction and stainability of the SAT-zone, in that the more contracted this zone is, the deeper it stains.

It is evident that the secondary constrictions in *Polygonatum* are nucleolar constrictions. Firstly, they correspond in number with the number of nucleoli in the different species. Secondly, an actual connection may be seen at prophase between the nucleolus and a number of chromosomes equal to the number of chromosomes provided with this kind of constrictions. This is especially clear in the pollen grain divisions. In Fig. 1 we see a prophase of the first pollen grain division in *Polygonatum officinale* (Piikkiö). Here, ten chromosomes are clearly discernible, one longer and one shorter chromosome being attached to the nucleolus. This is just what may be expected, as the species in its haploid set has one long and one short chromosome provided with secondary constrictions. The same state of affairs has been found by MATSUURA (1935) in the two *Polygonatum* species studied by him.

The somatic chromosomes of the liliaceous plant species *Smilacina stellata*, which is closely related to *Polygonatum*, have been examined for the sake of comparison. In Fig. 2 the somatic complement of this plant with 2n = 36 chromosomes is illustrated. *S. stellata* has two SAT-chromosomes resembling some types in *Polygonatum*. They are to be seen in the middle of the plate at the 9 and 10 o'clock positions. In addition, a number of SAT-chromosomes have been drawn separately from different cells (Fig. 3). In all of them a faintly stained, broader or narrower, region is observed in the hiata created by the secondary

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Figs. 2—3. *Smilacina stellata* (Helsinki). — Fig. 2. Somatic metaphase plate from a root tip (2n = 36). The two SAT-chromosomes in the middle at the 9 and 10 o'clock positions (B, CV—F). — Fig. 3. Somatic SAT-chromosomes drawn from different cells (2BE, F).

constrictions. Evidently also in *Smilacina* the constrictions are nucleolar constrictions, as the maximum number of nucleoli found is two.

The same mode of representation has been used in regard to a number of *Polygonatum* species in that the various types of SAT-chromosomes have been drawn separately from different cells (*cf.* Figs. 4—8). Reference may also be made to the figures represented in the earlier paper (SUOMALAINEN, *l. c.*), in which complete plates are drawn and the SAT-chromosomes may be compared with other chromosomes in the same complement.

It has been mentioned above that *P. officinale* has two pairs of chromosomes provided with secondary constrictions, one of the longest

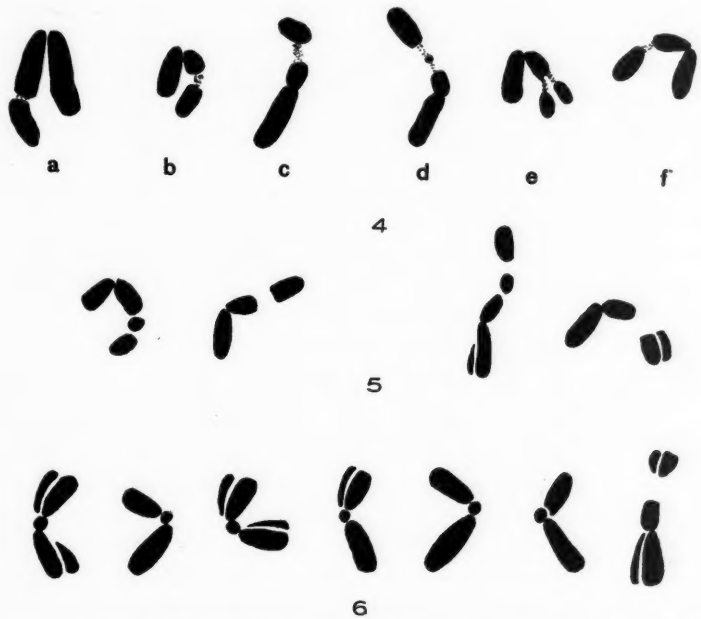


Fig. 4 *a—f*. *P. officinale* (Piikkiö). Somatic SAT-chromosomes drawn from different cells (Craf, CV). — Fig. 5. *P. officinale* (Skutskär). The SAT-chromosome pair drawn from two different cells (2BD, F). — Fig. 6. *P. sibiricum* (Gothenburg). Somatic SAT-chromosomes drawn from different cells (2BD, CV).

and one of the shortest chromosome pairs being nucleolar chromosomes. In Fig. 4 *a* the long chromosome is seen, while *b—f* represent the shorter one. The chromosome in Fig. 4 *d* exhibits a deeply stained body in the middle of the constriction, which phenomenon is not very rare. The slides from which the chromosomes in Fig. 4 have been drawn have been fixed in the Craf-solution, which as a rule renders the secondary constrictions more stainable than do other fixatives.

One strain of *P. officinale* from Skutskär (Sweden) has a small SAT-chromosome pair that is heteromorphic in regard to the structure of the constriction. The one of these chromosomes is of the normal type found in the other *P. officinale* strains studied, while the other member has a deeply stained segment in the middle of the constriction (Fig. 5). Inequality in a chromosome pair has more often been found in regard to the satellite-chromosomes than in other chromosome types. This does not, however, necessarily imply that members of SAT-

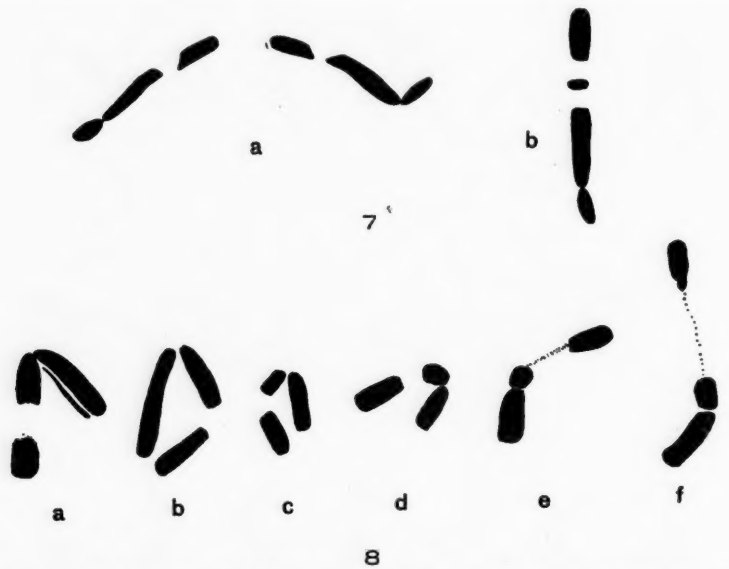


Fig. 7 *a*. *P. multiflorum* (Helsinki). A divided SAT-chromosome in anaphase side view (B, CV). — Fig. 7 *b*. *P. multiflorum* (Turku). A somatic SAT-chromosome. Note the stained body in the middle of the constriction (2BD, CV). — Fig. 8 *a—f*. *P. japonicum* (Weibullsholm). Somatic SAT-chromosomes drawn from different cells (*a*, *e*, *f* squash-preparation AA, F; *b* 2BD, F; *c*, *d* 2BD, CV).

chromosome pairs would more often be dissimilar than other chromosomes, as it must be remembered that differences are much more easily detectable in them than in other chromosomes.

Previously (SUOMALAINEN, *l. c.)* the chromosome complement of *P. sibiricum* (Gothenburg) was represented as containing two pairs of chromosomes with long nucleolar constrictions and one pair with less pronounced non-nucleolar constrictions. A closer examination of the nucleoli, the number of which seems to be six, has, however, made it evident that this last-mentioned chromosome pair also must be regarded as nucleolar chromosomes. Fig. 6 shows a number of representatives of the latter type (H-chromosomes) as compared with a SAT-chromosome of the more common type (at the right).

In Fig. 7 *a* two sister SAT-chromosomes in *P. multiflorum* (Helsinki) are seen in anaphase side view. Though the secondary constrictions in this case appear as unstained gaps, the behaviour of the chromosomes, in my opinion, indicates that the unstained segment must be of almost

the same breadth and rigidity as the other parts of the chromosomes. An interesting case speaking in favour of the same view is illustrated in Fig. 7 *b*. Here the secondary constriction includes a middle segment which is as broad and as intensely stained as the other parts of the chromosome. If the chromosome segments on both its sides had been thin, unspiralized threads, this segment ought to have acquired a rounded appearance of less diameter than the other chromosome parts, in accordance with the behaviour exhibited by the small terminal satellites.

The exceptionally long secondary constrictions of *P. japonicum* (Weibullsholm, Sweden) have been referred to already in my previous paper. In Fig. 8 a number of these SAT-chromosomes are presented. Especially elongated are the constrictions in the squash preparations (Fig. 8 *a*, *e*, *f*). We may, however, notice that even in these cases in which the secondary constriction has undergone such an extension, this region is often faintly stained. If a thin unspiralized thread had been so extended, it most certainly ought to have been broken.

To render the possibly present heterochromatin visible in *Polygonatum*, several species were subjected to cold-treatment for 3 and 6 days respectively. No nucleic acid starvation could, however, be observed in any of these cases. Neither could any chromocentres be discovered in the resting nuclei. Pachytene stages in the species in which meiosis has been studied (SUOMALAINEN, *l. c.*) did not, either, exhibit any heterochromatic segments in the form of more deeply stained or diffuse chromosome parts.

Though the cold-treatment did not reveal any heterochromatin, it had other effects on the chromosomes. As usually observed, also in the present case this treatment contracts the chromosomes, making them shorter and thicker as compared with untreated chromosomes. The secondary constrictions contract approximately in the same proportion. Often, especially in the longer chromosomes, they are so narrow as to be almost undetectable (Fig. 9 at the 8 and 9 o'clock positions). At the same time as the secondary constrictions are shortened the faintly stained SAT-zones often become broader and more deeply stained. Especially typical are the two chromosomes in Fig. 10 *e*—f, in which the constrictions are very narrow and relatively deeply stained.

Typical terminal satellites have been regarded as permanent chromosome organs comparable with any of the others, *viz.* primary and secondary constrictions, centromeres, and so on. This opinion is, indeed, supported by most observations made on satellites in various organisms, in which their occurrence and behaviour are as constant as that of any

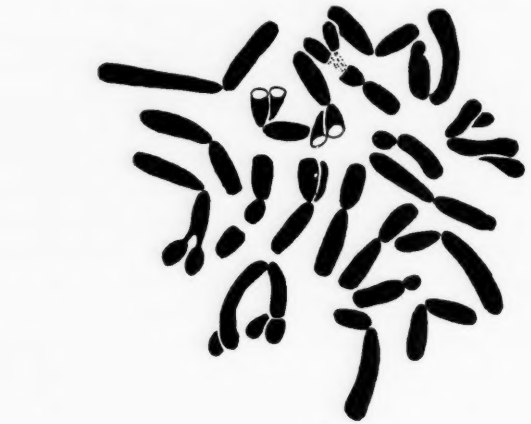
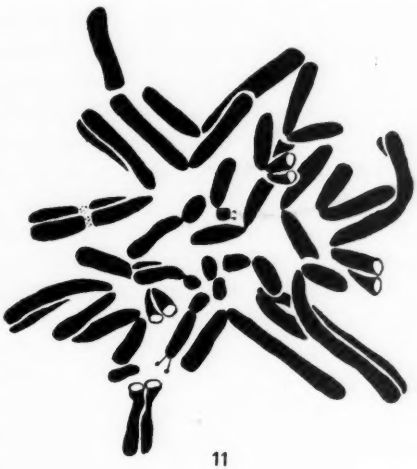


Fig. 9. *P. officinale* (Piikkiö). Somatic metaphase plate from a root tip (2n = 20). Cold-treatment 6 days. The long SAT-chromosomes at the 8 and 9 o'clock positions, the short ones at the 9 and 12 o'clock positions (B, CV). — Fig. 10 *a—e*. Cold-treated somatic chromosomes. — Fig. 10 *a—d*. *P. officinale* (Piikkiö). Cold-treatment 6 days (B, CV). — Fig. 10 *f—e*. *P. multiflorum* (Sweden). Cold-treatment 3 days (B, F).

other chromosome segment, changes in them being due only to structural re-arrangements. Certain exceptions to this general rule have, however, been recorded. For instance, SWEZY (1935) has found, in eight of the ten *Crepis* plants studied by her, an abnormal behaviour of the satellite, which appeared attached to different chromosomes of the complement in different plates. The author explains this as being caused by translocations. A reverse phenomenon to the known amphiplasty (*cf.* NAVASHIN, 1934) has been detected by LEVAN (1937) in an *Allium* hybrid. This hybrid possessed satellites, whereas the parental species lacked them. From these data LEVAN (*l. c.*, p. 346) concludes: »This appears to me to be compatible only with the assumption that

the satellites are manifestations of a genetic reaction and have nothing to do with simple structural changes».

An irregularity belonging to this group was encountered in *P. officinale* (Piikkiö). One plate in an otherwise normal root tip revealed, in addition to the usual secondary constrictions, two chromosomes with supernumerary terminal satellites (Fig. 11 at the 7 and 12 o'clock positions). The satellites are quite distinct in this plate, although they could not be detected in any other case in spite of the fact that a great number of excellent plates were available of this plant. The occurrence of the exceptional satellites may be due to an abnormal physiological condition of the cell concerned, since this cell contained 21 chromosomes instead of the normal 20. It is not known owing to what irregularity the cell has received the extra chromosome. A similar phenomenon has been found by MEURMAN and THERMAN (1939) in *Clematis Jackmanni*, in which one sporadical cell with a supernumerary satellite, not present elsewhere, could be observed.



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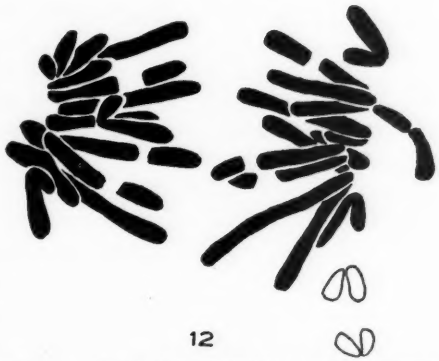

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Fig. 11. *P. officinale* (Piikkiö). An abnormal somatic metaphase plate with $2n = 21$ chromosomes. The chromosomes at the 7 and 12 o'clock positions are provided with exceptional satellites (2BE, CV—F). — Fig. 12. *P. multiflorum* (Sweden). Somatic anaphase from a root tip with a supernumerary small chromosome, drawn in outline (2BD, CV).

A mitotic irregularity of another kind has been observed in *P. multiflorum* (Sweden). In this plant one sector of a root tip contained

in its cells a supernumerary chromosome. This chromosome (Fig. 12, the chromosome drawn in outline) is of smaller size than any member of the normal complement. It seems also to be more contracted and more deeply stained as compared with the others. In a number of divisions it behaved fairly regularly, being, however, often delayed in its division. In other cells, again, it could be seen in some way unorientated outside the metaphase plate. The same inability to orientate itself is often expressed in anaphase in that this chromosome seems to divide quite irrespective of the spindle (Fig. 12). The appearance and behaviour of this supernumerary chromosome resembles closely what has been established for the supernumerary heterochromatic B-chromosomes in *Sorghum* (DARLINGTON and THOMAS, 1941), in *Zea* (DARLINGTON and UPCOTT, 1941), and in *Anthoxanthum* (ÖSTERGREN, 1947). Whether this supernumerary chromosome in *Polygonatum* belongs to the same group, and what its actual origin is, being found in one root tip only, cannot be decided on the basis of the present material.

IV. DISCUSSION.

1. HETEROCHROMATIN.

Heterochromatin — its structure and function — has been in the focus of most intensive research during the last few years. Cytology, genetics and chemistry have shed new light on this complicated problem from different viewpoints. The discovery of the decisive rôle played by the nucleic acids in the structure, reproduction and activity of chromosomes has been one of the chief results of these investigations. We may refer only to the following excellent reviews considering these questions from different points of view: GULICK (1941), DARLINGTON (1942, 1947), MIRSKY (1943), PONTECORVO (1944) and CASPERSSON (1947).

For knowledge of the chemical structure of the chromosomes and especially heterochromatin we are chiefly indebted to CASPERSSON and his collaborators (*cf.* CASPERSSON, 1939, 1940 a and b; CASPERSSON and THORELL, 1941; CASPERSSON and SANTESSON, 1942). The normal chromosome cycle is accompanied by the attachment and detachment of thymonucleic acid to and from the chromosomes. The maximum charge, as known, is reached at metanaphase. During telophase the acid gradually disappears and is practically absent in the resting nucleus, being retained only by the positively heterochromatic chromosomes. At the same time

the protein framework of the chromosomes undergoes a series of changes. At metaphase the proteins of the chromosomes are mainly of the histone type. During telophase and interphase the euchromatic chromosome parts produce proteins of higher globulin type, while heterochromatin excretes histones, of which a part forms the nucleolus. In prophase, again, the higher proteins break down and the nucleolus disappears. CASPERSSON and SANTESSON (1942, p. 13) have briefly summarized the results obtained in regard to the rôle of heterochromatin in the cell metabolism as follows: »The nucleus of the cell is a center for the formation of protein. This takes place through the mediation of nucleic acids which are of ribodesose type in the case of the gene-carrying chromosomes and of ribose type elsewhere. The heterochromatic portions of the chromosomes regulate this metabolism and during the interphase seem to build protein substances of a type rich in hexone bases. These form the main part of the nucleolus and wander out toward the nuclear membrane. At this surface are built ribose nucleic acids and, apparently by their mediation, the cytoplasmic proteins.» It must, however, be borne in mind, as pointed out by CASPERSSON and SANTESSON (*l. c.*), that heterochromatic regions, having the chemical structure and function described above, are in most cases very difficult to identify cytologically. For this reason CASPERSSON (1947) has later proposed to call these chromosome parts by the non-committal name nucleolus-associated chromatin to avoid the use of the complex term heterochromatin.

The cytological side of problems connected with heterochromatin has been especially elucidated by the studies of DARLINGTON and LA COUR (1938, 1940, 1945). They discovered that in a number of liliaceous plants heterochromatic segments could be made visible with cold-treatment in that the plants were kept in a temperature near the freezing point for several days. After this treatment the differential segments contained less thymonucleic acid at metanaphase as compared with the other chromosome parts. They were clearly narrower and understained with Feulgen and other chromatin stains. In the resting nuclei these segments showed a reversed behaviour, appearing as overcharged chromocentres. The most important feature which distinguishes these parts cytologically from other chromosome segments seems, indeed, to be their reversed thymonucleic acid cycle. This differential behaviour in its most typical form has been observed, *e. g.*, in *Trillium*, *Paris* and *Fritillaria* (DARLINGTON and LA COUR, 1938, 1940), in *Adoxa* (GEITLER, 1940), and in *Triton* (CALLAN, 1942).

If we consider the various phenomena which the term heterochromatin comprises — positive and negative heteropycnosis, positive and negative heterochromacy — one common denominator, to use DARLINGTON's expression, is the differing thymonucleic acid charge in the heterochromatic regions. These regions may be undercharged or overcharged during the whole nuclear cycle, but the most typical case seems to be that their thymonucleic acid cycle is reversed, or as expressed by KLINGSTEDT (1941, p. 172): »A displacement of the timing processes, in such a way that the heterochromites go through about the same cycle as the euchromites but more or less out of step with the latter, would seem to account for all the known facts better».

As pointed out above, the cytological identification of heterochromatin is often very difficult. This may depend on the fact that heterochromatic segments may be very small and scattered amongst euchromatin or on their variable behaviour in different circumstances. The same heterochromatic segment may in certain conditions show allocycly, in others again not. In regard to the heterochromatin in *Paris* species DARLINGTON (1941) has come to the conclusion that allocycly is genetically controlled in that the same chromosome parts which behave allocyclically in one species do not do so in another.

The hypothesis of HEITZ (1932) that heterochromatic regions are genetically inert has found support in the fact that these chromosome parts have been found to contain no genes in the usual sense of the term. It has, however, become evident that heterochromatin is not inactive, but has a profound significance in cell metabolism. Genetical studies on the function of heterochromatin have given rise to MATHER's (1944) concept of polygenes, which differ from the real genes in having small, similar and supplementary effects. They are more unspecific as compared with the other genes. In the light of the knowledge of the chemical structure and function of these parts this might be thought to depend on the fact that heterochromites secrete the less specific and simpler histones, whereas euchromatin produces the highly specific gene proteins. It must, however, be stressed that the concepts of heterochromatin arrived at from different points of view apparently cover each other only partly.

2. SECONDARY CONSTRICTIONS.

The term »secondary constrictions» has been applied to all those constrictions in the chromosomes which do not contain the centromere. Secondary or SAT-constrictions have been found in most plants and

many animals in one form or another (literature summarized in RESENDE, 1937, 1940). HEITZ's (1931 a and b) discovery that the nucleoli arise at the secondary constrictions has established an important function of the latter.

Secondary constrictions may be intercalary or almost terminal, in the latter case often separating a typical small satellite or trabant from the main body of the chromosome. The satellites are attached to the chromosomes by means of a more or less visible, thin satellite thread. It is known that constrictions are points of weakness in the chromosome. Chromosomes are mostly bent at these regions, and if they are exposed to stress these parts most easily yield and finally break. Constrictions are more labile in their appearance and behaviour than other chromosome parts, especially their length being subject to variation. Except that the nucleoli in most plants are formed at the secondary constrictions, the other possible functions of the constrictions are so far obscure. There also exist secondary constrictions which seem to have nothing to do with the formation of nucleoli.

Attempts have not been wanting to interpret secondary constrictions as some kind of heterochromatic regions. DARLINGTON and LA COUR (1940) have represented the view that all secondary constrictions except those due to nucleolar organizers may be heterochromatic chromosome regions. KLINGSTEDT (1941, p. 173), on the other hand, has put forth the following suggestion: »One may perhaps tentatively suggest that the nucleolus organizer is a special kind of heterochromite, the productive activity of which is postponed until telophase and consists in a condensation of chromosome products so much changed as not to show the staining reactions of thymonucleic acid any more». Indeed, if we define heterochromatic chromosome regions in terms of a reversed or changed thymonucleic acid cycle, all secondary constrictions, nucleolar as well as non-nucleolar, evidently are heterochromites. This view is supported by the fact that there are a great number of cases which seem to be intermediate between typical heterochromatin in the sense of DARLINGTON and LA COUR (*l. c.*) and typical secondary constrictions, and which might with equal justification be included in either group of structures. Examples of this are offered by a great number of plants with excessively numerous secondary constrictions. For instance, many of the arctic *Ranunculus* species studied by FLOVIK (1936) exhibit such constrictions. Similarly, *Lachenalia* species (MOFFETT, 1936) have a very great number of secondary constrictions, which also resemble heterochromites in so far as certain segments are much narrower than

others. It is also difficult to decide how to classify the heterochromatic segments found by LEVAN (1942) in the cold-treated haploid rye. If we bear in mind that timing differences in the thymonucleic acid cycle are a characteristic feature in the behaviour of heterochromatin, the numerous secondary constrictions in the chromosomes of the Salmonoids (SVÄRDSON, 1945) are of special interest. They are clearly visible during somatic prophases, at which stage they appear as narrow and understained segments. Gradually, however, they catch up the other chromosome parts, being at full metaphase indistinguishable from the latter.

As known, the nucleolar organizers in plants usually lie in secondary constrictions, while those in animals are often situated in heterochromatic regions. Since the cytological identification of heterochromatin has in many cases turned out to be impossible, CASPERSSON (1947), as mentioned above, has proposed for this material the term nucleolus-associated chromatin. This name would, according to its definition, also apply to the chromatin of the secondary constrictions in plants.

As opposed to the view that secondary constrictions belong to heterochromites, RESENDE (1940) has presented the opinion that the heterochromatic regions of DARLINGTON and LA COUR are in reality secondary constrictions. Though this controversy to a great extent is a matter of definition, the above view seems to be more plausible as compared with RESENDE's hypothesis, since the term heterochromatin according to its current use is a wider concept than the secondary constriction.

In regard to the structure of secondary constrictions DARLINGTON (1937, p. 39) has given the following definition: »All constrictions are regions of the chromosomes which fail to undergo spiralisation along with the rest». In many cases, especially in terminal satellites, the so-called satellite thread is in fact stained and visible. HEITZ (1931 a, p. 89) has, however, already pointed out that the satellite thread, although stainable with other methods, often does not show the Feulgen reaction. From his figures it is seen that in many cases the »thread» is equal in breadth to the main chromosome body, its staining being, however, so faint as to be almost invisible. In most papers dealing with secondary constrictions a thin satellite thread has been represented. It is, however, impossible to draw any conclusions from this, as it may be only a matter of conventional drawing and not express the actual state of affairs. In passing it may be mentioned that the same doubt attaches to the representation of the segments separated by secondary

constrictions. They have often been drawn as rounded bodies like beads in a string (*cf.*, *e. g.*, FLOVIK, 1936; MOFFETT, 1936), while in other cases the segments have clear-cut edges and thus exhibit the shape of rectangles (*cf.*, *e. g.*, HEITZ, 1931 a; ÖSTERGREN, 1947).

It must in this connection be taken into consideration, as especially stressed by ELVERS (1943, p. 200), that structures approaching the limit of the resolving power of the microscope do not give true images, the limit for true reproduction with the ordinary microscope being 1,5—2,0 μ. It is evident that this makes the exact interpretation of structures smaller than this fairly doubtful. In addition, bodies which are too small as compared with the wave-length of light will appear rounded. ELVERS (*l. c.*) thinks that this might account for the rounded appearance of chromosomes in general and especially of the small satellites.

The question of the nature of secondary constrictions has been considered especially by RESENDE (1939 a and b, 1940). In a number of unrelated phanerogamous species he observed that the SAT-thread was not a thin threadlike structure, but almost as broad as the main part of the chromosome. RESENDE (1940) therefore suggests the term SAT-band (SAT-zone) for these regions. The behaviour of the SAT-zones in his material was very labile in most respects. In a single plant their length varied from cell to cell, the two homologues even in the same cell being often divergent. The same was true of the Feulgen reaction, the same chromosome type showing all degrees from an unstained gap to a region which was almost as intensely stained as the other chromosome parts. RESENDE (1940) claims to have observed a spiral structure in all parts of the chromosomes, including the SAT-zones. This finding is explained by him on the assumption that the amount of thymonucleic acid and consequently the degree of spiralization are very variable in the constrictions.

The present observations on *Polygonatum* and *Smilacina* agree in many respects with RESENDE's findings. Also in this case the secondary constrictions appear either as unstained hiata, or if stained they do not as a rule reveal a thin satellite thread, but a broad zone, often equal in breadth to other chromosome segments. It is true that in neither of these genera is a spiral structure directly observable in the constrictions. The following facts seem in spite of this to speak in favour of its existence. When stained the SAT-zone is often almost as broad as the main part of the chromosome. It is difficult to understand how an unspiralized thread would give such an image. In squash preparations

(*e. g.*, Fig. 8 *e, f*) the constrictions are often much extended. The fact that the SAT-thread despite this is still visible might, in my opinion, be accounted for by assuming that an originally prevailing spiral structure has been uncoiled by the stress. For the second, cold-treatment contracts these parts almost proportionally to other chromosome segments. Since this contraction of the chromosomes depends on a more compact spiralization, we might in analogy suppose the same to be true of the constrictions.

A further argument in favour of this view is that certain segments separated by the secondary constrictions in *Polygonatum* and *Smilacina* have the shape of rectangles instead of rounded bodies, which they apparently ought to have if the SAT-thread should be unspiralized. In addition, the mechanical behaviour of constrictions at cell divisions (*cf.* the anaphase side view in Fig. 7 *a*) would suggest that the breadth and rigidity of these parts, even when they are unstained, are equal to the ones of the main part of the chromosome. Finally, an intercalary segment in the middle of the constriction may appear as a stained body (Fig. 7 *b*), which may be as broad as the other chromosome parts, and not a rounded small body similar to the tandem satellites described in the literature.

It must be stressed that the above observations do not imply that the SAT-zones are spiralized to the same degree or as regularly and compactly as the other chromosome parts. It is quite evident that just as their thymonucleic charge has been observed to vary, so does their spiralization show a wide range of variation.

The explanation of the undercharging and underspiralization of the secondary constrictions has been sought in the fact that at prophase these regions are associated with the nucleolus which would prevent their charging at the same time as other chromosome parts (*cf.* MENSINKAI, 1939). RIBBANDS (1941), again, has suggested that the nucleolar substance and euchromatin exert a mutual repulsion, which would result in the formation of the hiata of the secondary constrictions.

The assumption that the satellite thread is more or less spiralized, being possibly completely unspiralized in the case of the terminal satellites, would in my opinion explain a number of known phenomena. For instance, it might be thought that the sporadical occurrence of satellites reported in certain plants (*e. g.*, SWEZY, 1935; MEURMAN and THERMAN, 1939) would depend on the uncoiling of a number of gyres in the chromatid spiral. This again might be accounted for by some physiological change in the cell concerned. The same interpretation

may also apply to the exceptional cell in *Polygonatum officinale* (Fig. 11) with 21 chromosomes in which two of the chromosomes are provided with exceptional satellites. The known phenomenon of amphiplasty might also be interpreted in the same terms, the more so as NAVASHIN (1934) has stated that in the *Crepis* hybrids showing amphiplasty the volume of the satellite-bearing arm increased simultaneously with the disappearance of the satellite. LEVAN (1937) has in an *Allium* hybrid observed the reversed phenomenon to amphiplasty, the hybrid exhibiting a satellite, which is not found in the parental species. He explains this to be due to a differential contraction of the SAT-thread, or in other words to its differential spiralization.

The relationships of the thymonucleic acid charge of a chromosome and its spiralization have been much discussed, and in most of the theories concerning the mechanism of the spiralization thymonucleic acid has been ascribed a prominent rôle. The attachment of thymonucleic acid to the chromosomes during prophase and their spiralization are indeed parallel phenomena. Further it has been established that the more heavily a chromosome is charged with thymonucleic acid the more compactly it is spiralized. This is especially clearly seen in the positively heteropycnotic chromosome parts, which during the resting stage retain their thymonucleic acid charge (*cf.* WHITE, 1940). An interesting example is furnished by the golden hamster (KOLLER, 1938), in which the *X* chromosome consists of a positively heteropycnotic pairing region which is overcharged with thymonucleic acid and compactly spiralized and a negatively heteropycnotic differential region which is undercharged and almost unspiralized. The heterochromatic regions in *Trillium* and *Paris* also represent several types, from more darkly stained and spiralized to faintly stained and unspiralized segments. DARLINGTON and LA COUR (*cf.* DARLINGTON, 1942; DARLINGTON and LA COUR, 1945) are therefore of the opinion that spiralization is directly caused by the attachment of thymonucleic acid to the chromosomes. CALLAN (1942) came to the same conclusion when studying the behaviour of heterochromatin in the cold-treated *Triton*. In this animal the heterochromites possess a spiral structure in mitosis when thymonucleic acid is available, while spiralization fails completely in meiotic metaphase owing to the lack of nucleic acid in the preceding pachytene. CALLAN accordingly concludes (1942, p. 332): »We may in fact suppose that, while heterochromatin may or may not assume a spiral state, if it is utterly deficient in nucleic acid throughout the cell cycle, it cannot spiralize». It is difficult to contradict this statement, as it is almost

impossible to decide when a chromosome region is »utterly deficient in nucleic acid». It must also be borne in mind that the staining reactions by means of which heterochromatic regions are rendered visible depend as a rule on the presence of thymonucleic acid. If nucleic acid is lacking no conclusions can therefore be drawn in regard to the structure of the heterochromites, at least with the ordinary microscopical methods.

In contradistinction to the views described above, CASPERSSON (1939) thinks that the nucleic acid synthesis is completed before the chromosomes begin to contract in meiosis, the amount of nucleic acid being constant from middle leptotene to diplotene, during which period the chromosomes actually undergo spiralization. The contraction, *i. e.* spiralization, of the chromosomes would thus, according to CASPERSSON (*l. c.*), take place independently of the nucleic acid synthesis.

This question needs, however, further consideration before it can be regarded as finally solved, especially as the exact mechanism of the spiralization process is not known. An established fact is that, even if the attachment of thymonucleic acid to the chromosomes should not be the direct cause of spiralization, the thymonucleic acid charge locks up the spiral structure, giving compactness and rigidity to it. This is especially clearly proved by the labile behaviour of those chromosome parts in which the thymonucleic acid charge is deficient.

In plants the nucleoli arise with but few exceptions at secondary constrictions, while in animals they are in most cases formed in connection with the heterochromites. MC CLINTOCK (1934) has in her classical paper on maize described the nucleolar organizers as specific bodies which stain intensely in aceto-carmine and which are situated next to the regions where the nucleoli and secondary constrictions appear. In accordance with this DARLINGTON (1942, p. 66) has defined nucleolar organizers as follows: »Thirdly the nucleoli usually arise next to one or more particular chromomeres, the nucleolar organizers which are again compound genes breakable by x-rays, and which may be either at the ends or in the middle, either in a heterochromatic or euchromatic part of the chromosomes.»

The rôle ascribed by CASPERSSON (1940 a) to the nucleolus in the nucleic acid and protein metabolism of the cell has been briefly described above. The old theory that the nucleoli form a source of reserve material for the building up of »chromatin» at prophase has been revived by CASPERSSON (1940, p. 595), who has suggested that nucleoli are used as material for the thymonucleic acid synthesis. It is in fact true that the compounds which would be best suited to form

the pyrimidine and purine groups of thymonucleic acid, are arginine and histidine, the high concentration of which just characterizes the histones, which in turn form the main part of the nucleolus.

It has become evident that there exists some kind of relationship between the nucleolar system and heterochromatin, though its true nature is still obscure. In species belonging to the genera *Paris*, *Trillium* and *Fritillaria*, the presence of well-developed heterochromatic segments seems, but for a few exceptions, to be incompatible with that of nucleolar organizers (DARLINGTON, 1941). In other organisms, however, heterochromites and nucleolar organizers have been found to co-exist, while in still others both seem to be lacking (*cf.* DARLINGTON, 1947). DARLINGTON (*l. c.*) has explained this to depend on the fact that the nucleolus and the charged heterochromatin in the resting stage would be alternative sources of nucleic acids, which are used at prophase when the chromosomes are charged with thymonucleic acid. An excess of heterochromatin on the other hand, as in the *Sorghum* plants provided with extra heterochromatic B-chromosomes (DARLINGTON and THOMAS, 1941), seems to upset the nucleolar apparatus in that the nucleoli do not dissolve at prophase, but remain to later stages. It may be of relevancy in this connection that in *Polygonatum* and *Smilacina*, where the nucleolar constrictions are so exceptionally well-developed, no trace of typical heterochromatin has been observed either as understained segments after cold-treatment, or as chromocentres in the resting nuclei, or as diffuse regions at pachytene.

Many questions arising in connection with the secondary constrictions are so far unsolved. It is not known whether all secondary constrictions are homologous in principle, nor whether there exist different kinds of them. Neither it is clear what the true nature of the difference between nucleolar and non-nucleolar constrictions is. It also remains to be discovered whether there is an actual difference between constrictions with spiralized and completely unspiralized SAT-threads, and what its significance would be. The relationship between typical heterochromatic regions and secondary constrictions is also so far obscure, though these structures exhibit many related features and both seem to be connected with the nucleolar apparatus. These problems ought to be subjected to investigation, using as material, if possible, some organism provided with heterochromites as well as with different kinds of secondary constrictions.

I wish to acknowledge my gratitude to the Director of the State Horticultural Institute, Professor O. MEURMAN, for the opportunity to carry out this study at the Cytological Laboratory of the Institute.

SUMMARY.

(1) The long and well-developed secondary constrictions in several *Polygonatum* species belonging to the group *Alternifolia* and in *Smilacina stellata* have been studied.

(2) These secondary constrictions appear as clear-cut gaps in the chromosomes. The intervening region is very labile both as to its staining and its contraction reactions. When stained, it does not have the appearance of a thin thread, but is almost as broad as the other parts of the chromosome.

(3) The secondary constrictions are true nucleolar constrictions in *Polygonatum* and *Smilacina*, agreeing in number with the nucleoli. Fûrther, a connection between the nucleolus and the expected number of chromosomes has been observed at the prophases.

(4) The nature of heterochromatin is discussed, and secondary constrictions are regarded as negative heterochromites in the widest sense of this concept.

(5) A spiral structure of the SAT-zones, though not actually visible, is inferred from (a) their breadth, when stained, (b) their contraction, when subjected to cold-treatment, and (c) their general appearance and behaviour.

(6) *Polygonatum* and *Smilacina* do not show any trace of typical heterochromatin after cold-treatment or otherwise, as understained segments at metanaphase, chromocentres at resting stage, or diffuse regions in pachytene.

(7) The labile length and staining properties of secondary constrictions are explained in accordance with RESENDE (1940) on the basis of their variable thymonucleic acid charge and the accompanying variation in their spiralization. Amphiplasty and related phenomena are considered to be explicable on the assumption that the presence or absence of a satellite depends on the uncoiling or coiling of a number of gyres in the spiral.

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ABSTRACTS — KURZE MITTEILUNGEN

BENGT KIHLMAN and ALBERT LEVAN: The cytological effect of caffeine.

The action of methyl xanthines, especially that of caffeine, on mitosis and chromosomes has been studied by several workers (*e.g.*, GOSSELIN, 1940; DEYSSON, 1948). Two features in its action have been mainly observed: (1) the formation of cell-walls after mitosis is impeded, although the spindle functions normally, and (2) chromosome-bridge formation at anaphase occurs. FRIES and KIHLMAN (1948) found a mutagenic effect of caffeine and theophylline in the ascomycete *Ophiostoma multiannulatum*. The present work gives a preliminary report on the cytological effect of caffeine, as studied according to the *Allium* test (cf. LEVAN, 1949).

1. *Lethality, mitosis-depression, c-tumours and c-mitosis.* — Caffeine has a lethal action in strong concentrations (1 and 2 %). Even lower concentrations have a suppressive action on the frequency of mitoses. Thus, 24 hours' treatment with 0,4 % gives total suppression of mitosis. In 0,2 % single mitoses occur, while 0,1 and 0,04 % only effect some depression. From 0,02 % downwards the number of mitoses is normal or even higher than normal. The mitosis-free period after 24 hours' treatment with 0,4 % is about 12 hours. C-tumours are found in the concentration zone 0,02—0,2 %. Caffeine and other methyl xanthines tested do not induce full c-mitosis. Very strong concentrations, though, may show clear c-mitotic tendencies (*e. g.*, 1 % caffeine and 1 % theophylline).

2. *The failure of cell-wall formation* (the reaction of »stathmodiérèse» of French workers) is very characteristic of caffeine. It results in the origin of, firstly, binuclear cells and, later, of cells with more nuclei. The reaction has been observed as early as after half an hour's treatment and gradually involves 100 % of the dividing cells. The threshold of this reaction lies between 0,02 and 0,04 %. In the latter concentration cell-wall formation is often checked, but the reaction is not complete.

This reaction has remarkable cytological consequences. Cells will arise with a high number of chromosomes. During mitosis the various nuclei in each cell will often fuse, giving rise to large clusters of chromosomes. Sometimes the two nuclei of a binucleate cell are arranged in tandem, resulting in four anaphase groups. The two groups in the middle are pushed together from both sides and will often form one telophase nucleus. Thus, a characteristic picture seen in long cells at a certain stage of treatment is the following: one diploid nucleus at each end of the cell and one large tetraploid nucleus in between. After prolonged treatment the tissue will assume a striking appearance, small diploid cells being mixed with all kinds of polyploid cells and the tissue organization being very much disturbed. In such a tissue mitoses showing great disturbances and considerable variation in chromosome number and direction of spindle axis are frequent. A close analysis of this development will be undertaken.

3. *Influence on the chromosomes.* — The main object of the present study is to look into the chromosome changes which have been reported from

caffeine treatments. We have found that caffeine induces structural chromosome changes and in this respect is comparable to mustard gas and radiations.

Chromosome changes have been observed in the concentrations 0,02—0,4 %. We have tried to distinguish between »primary» and »secondary» changes, *i. e.* what LEA (1946) classifies as »physiological» and »structural» changes respectively. It is quite characteristic that the chromosome changes seen directly after the start of the treatment are mostly such changes as may be due to stickiness of the chromosome matrix, the most common change being anaphase bridges. The difficulty of separation affects only one or a few of the chromosomes of each cell. The extreme stickiness obtained with ethylene glycol (ÖSTERGREN, 1944) involving connections between all separating chromosome arms has seldom been met with here. Another reaction appearing soon after the start of the treatment is the so-called pseudo-chiasma formation (LEVAN, 1949). Although the occurrence of this reaction in our experiments is evidently connected with the stickiness phenomenon it has a definite morphological type distinguished from the more non-specific stickiness. It indicates that certain points of the anaphase chromosomes are unable to separate.

The percentage of cells in mitosis with chromosome disturbances increases with increasing concentration up to the lethality zone. After a treatment of four hours in 0,04 % caffeine this percentage is about 5 %, after the same time in 0,2 % it is about 60 %, and in 0,4 % about 80 %. This correlation between concentration and effect, however, seems to be limited to the physiological changes. Concerning the structural changes we have not been able to observe such a simple correlation.

The structural changes, fragments and »real» bridges, commence to appear after some hours of treatment. Since the morphological type of these bridges is often indistinguishable from that of sticky bridges, the identification of the two types is rendered more difficult. Therefore, in some experiments the bulbs were transferred to pure water after 24 hours' treatment. After 6—12 hours in water the physiological type of changes ceases to occur, the remaining bridges being due to irreversible structural changes. Their character of translocation bridges becomes more certain the longer the period in water has been.

In these series, including 24 hours' treatment with 0,1, 0,2, and 0,4 % caffeine and a following treatment with water, we made rough estimations of the frequency of cells with structural chromosome changes. After the end of the treatment fixations were made during one week. All through this period structural changes were found, fragments, sister chromatid reunion, reciprocal translocations, *etc.* The highest frequency (in some cases more than 30 % of the total number of mitoses) was found after treatments with 0,1 %. In treatments with higher concentrations values exceeding 20 % were not recorded. In the 0,4 % series the changes present were generally of a more extreme type. Only here among all the series were pronounced cases of chromosome erosion (LEVAN and TJIO, 1948) met with.

4. *Some experiments on the influence of pH variation* on the action of caffeine were performed. The *pH* range 4,5—9,5 was covered in these experiments. No simple correlation between the *pH* of the solution and the frequency of induced chromosome disturbances was found.

5. *Other methyl xanthines tested.* — Some attention was also directed to the action of theophylline and theobromine. It was found that both of them had the same effect as caffeine on the cell-wall formation, the thresholds of this reaction being situated at about the same concentration in all three substances. Theophylline was also tested as to its effect on the chromosomes. As already pointed out by earlier writers, this substance has less visible effect in this respect. In our experiments fragments and translocations were recorded in the same concentration range as in caffeine (0,02—0,4 %).

The cyto-genetical work with purine derivatives varying in their content of alkyl- and oxi-groups will be continued.

Institute of Physiological Botany, Upsala, and Institute of Genetics, Lund, December 15, 1948.

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NILS OLSSON and CARL WADNE: A mutation in chicken.

In the offspring of Rhode Island red reared during 1946 a single pullet (No. 7794) was found, lacking flight feathers (primaries and secondaries) in its wings. The tail was normally feathered. To investigate whether this character was a modification or a hereditary character this pullet was mated (1947) with a normal male of the same breed. All the offspring from this mating looked normal and none lacked flight feathers. During the next season (1948) a male from these offspring was mated with its mother (No. 7794) and one of its sisters (No. 2585). From the old hen (No. 7794) 11 chicks were recorded of which two pullets had no flight feathers in their wings. From the pullet (No. 2585) mated in the same pen, 9 chicks were recorded of which one pullet lacked flight feathers. These results show that the character »no primaries and secondaries in the wings» is hereditary in nature and probably a recessive allelomorph to the normal factor for primaries and secondaries. Further investigation of the segregation is, however, desirable. The hereditary nature of this character has not been previously shown in Sweden. No dif-

a *b*

Fig. 1. Rhode Island red birds. *a*: with normal wings; *b*: lacking flight feathers (primaries and secondaries).

ference between the wings of normal pullets and those lacking primaries and secondaries was found in the chicks at 8 weeks of age. This indicates the presence of these feathers in the wings before the first moulting.

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HELGE JOHNSSON: Hereditary precocious flowering in *Betula verrucosa* and *B. pubescens*.

Both *Betula verrucosa* and *B. pubescens* flower as a rule for the first time at an age of between five and ten years. When making a survey of a very extensive cultivation of two-year-old birch plants in 1941 the author came across two *B. verrucosa* plants and one *B. pubescens* plant which at this exceptionally early time had already put forth flower-buds. Ever since then these individuals have flowered plentifully every year. In the spring of 1946 the two *verrucosa* plants were crossed with each other. The resulting seeds were sown in the greenhouse in February, 1947, the seedlings transplanted into frames in May the same year and planted out in open soil as one-year plants in 1948. This planting out in the open was effected in nursery beds with a distance of 25 cm between rows and 15 cm between plants. On completing growth the material, which comprises 659 plants, has reached a mean height of $130{,}4 \pm 1{,}81$ cm, which means an extremely good development and also signifies that the plants have stood rather crowded on account of the above-mentioned spacing. The mean number of living lateral branches is $17{,}51 \pm 0{,}44$. Like the parents, a high percentage of the progeny already produced staminate buds the second year, 462 plants having borne flower-buds on one or more lateral branches. Of these, 31 have produced catkins at the same time, which means that these plants have already developed pistillate buds during their first year of life. Among the 197 plants without male buds 2 have borne catkins. Hence the total number of plants which have developed florale buds before two years of age is 464, as against 195 non-flowering plants. This signifies that 70,41 % of the progengy have shown extremely early flowering.

In this there may lie a suggestion of a monohybrid segregation of a dominant factor for precocious flowering, in which case both the parent individuals should be heterozygous for this factor. If this is so, the flowering/non-flowering ratio would be 494,25 : 164,75 as compared with the 464 : 195 obtained. χ^2 for the agreement between *found* and *excepted* will be 7,4056, which corresponds to a probability of $0{,}01 < p > 0{,}001$ that the distribution found represents a monohybrid segregation. Thus, the monohybrid hypothesis seems to be but little probable if the deviation from the excepted distribution should depend solely on the random error. It is however likely that some plants have not developed flower-buds although in constitution they belong to the flowering group. The production of buds is obviously also dependent upon environmental factors, and then especially on exposure of the plants to solar light. As already mentioned, the material has grown up under dense conditions, and it is therefore conceivable that a number of plants have been so overshadowed that they have not budded. The extent to which budding takes place is also very varying among the flowering plants. As a measure of the abundance of flowering use may be made of the number of lateral branches per plant with a bud or buds, when the following figures are obtained:

Number of flowering lateral branches per plant	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Number of plants	56	27	31	23	32	23	23	24	26	23	25	18	19	25	13	16
Number of flowering lateral branches per plant	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
Number of plants	14	10	9	1	6	5	3	1	2	2	2	0	0	0	1	

This distribution intimates that a fraction with 0 flowering lateral branches ought also to occur within the flowering category. This becomes still clearer if the material is arranged in broader classes, *e. g.* with 5 lateral branches per class:

Class	0	1	2	3	4	5	6	7
No. of lateral branches	0	1—5	6—10	11—15	16—20	21—25	26—30	31—35
No. of plants	(32)	169	121	100	50	17	4	1
Expected	61	130	132	92	50	20	7	2

If it is assumed that the 32 individuals missing for a perfect 3 : 1 segregation are among the non-flowering plants, the flowering intensity would average 2,08 expressed in classes. A Poisson series with this mean gives the »expected» distribution stated. The agreement between the actual distribution and the Poisson series is not too poor. The mathematical law of distribution postulates that as many as 61 plants of those genotypically flowering would not have developed male or female buds owing to inadequate external conditions. If that is so, the ratio of 462 : 197 found will be changed to 523 : 136 and χ^2 reduced from 7,4056 to 6,6895, which increases the probability in favour of the 3 : 1 hypothesis a little though not much. There is however another method by which to estimate the number of genotypical flowering individuals without buds. Of the 462 plants with flower-buds, 31 simultaneously had catkins, *i. e.* 6,27 %.

Of the 197 plants without flower-buds, 2 had catkins. If the frequency of female flowering plants is the same in both the categories, 30 (29,81) plants of those not bearing buds will be genotypically flowering and the ratio will be 492 : 167 or very close to the expected 494,25 : 164,75. It accordingly seems probable that both the parent individuals are heterozygous for a dominant factor that produces flowering precocity.

The individual with premature flowering encountered in *B. pubescens* was crossed at the same time as the *verrucosa* cross was carried out with a normal *B. pubescens*. A small progeny of 128 individuals have been raised in the same way as was described for *B. verrucosa*. In this progeny 24 plants have developed flower-buds the second year and 104 not. Here, too, a dominant factor is indicated. In that case crossing with a recessive normal type should have given 64 : 64 instead of 24 : 104. Still, it may here have to be considered that *B. pubescens* is a tetraploid species ($x = 14$) and that the parent individuals may possess the constitution *AA/aa* and *aa/aa* with regard to precocious flowering. With random chromosome distribution the genotypic segregation would then be 1 *AA/aa* : 2 *Aa/aa* : 1 *aa/aa*, and if 1 *A* does not dominate over 3 *a* the phenotypic segregation would be 1 flowering : 3 non-flowering, or among 128 individuals 32 : 96 as compared with the found distribution of 25 : 104. $\chi^2 = 2{,}6667$ is found and the probability for agreement is $0{,}20 < p > 0{,}10$, *i. e.* very good.

The hereditary nature of the cases of precocious flowering found in *B. verrucosa* and *B. pubescens* is accordingly clearly demonstrated experimentally. In all probability it is a question in both cases of dominant major genes. Absolute certainty on this point must be left to future observations.

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